THE UNIVERSITY OF ALBERTA

# KINETICS OF CARBON DIOXIDE IN RUMINANTS

## An Evaluation of the Influence of Acidosis on the Carbon Dioxide Entry Rate Method for Estimatation of Rate of Energy Expenditure of Free Ranging Animals

by


KOU-JONG LIN


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## ABSTRACT

A recently developed technique for estimation of energy expenditure of field animals based upon measurement of carbon dioxide entry rate apparently worked satisfactorily for sheep and cattle at pasture. However, occasionally abnormally high entry rate values were observed. Following the published method for estimation of carbon dioxide entry rate, $NaH^{14}CO_3$ was infused at a constant rate intraperitoneally into sheep and cattle to establish - plateau equilibrium between the infused $^{14}CO_2$ and metabolically produced $CO_2$. The carbon dioxide entry rate (mg C/min) in the animal were estimated from the ratio of rate of infusion of $NaH^{14}CO_3$ (n ci/min) to the specific activity of $CO_2$ (n ci/mg C) in the samples of arterial blood, venous blood, expired gas, urine, rumen fluid and milk. The value of specific activity of $CO_2$ derived from the different body fluids were not the same. It was therefore concluded that for use of the carbon dioxide entry rate method it was necessary to establish individual regression equations between the actual energy expenditures (measured from the respiratory gaseous exchange) and carbon dioxide entry rate values estimated from the different body fluids. The regression relationship established based on the samples of arterial blood, venous blood, expired gas and urine were significant ($p < 0.01$).

Following development of acidosis in sheep, by the infusion of HCl into the rumen, the specific activity of $CO_2$ in the arterial blood, venous blood and expired gas were not markedly altered but a significant ($p < 0.01$) decrease occurred in the specific activity of $CO_2$ from urine. Analysis the kinetics of $CO_2$ showed that the pH of body fluids greatly influenced the $CO_2$ filtration and reabsorption in the kidneys. The $CO_2$ excretion rate in the urine was significantly ($p < 0.01$) decreased with a decrease in pH of the urine. The $CO_2$ excretion rate in the urine was reduced to extremely low levels (from 5.8668 to 0.0086 mg C/min) when the animal was changed from an alkalosis to acidosis condition.

It was estimated that about 0.0082 mg C/min of unlabelled $CO_2$ (from metabolism) was excreted by the cells of the collecting tubules and urinary bladder. This excreted unlabelled $CO_2$ apparently diluted the filtered $CO_2$ upsetting the equilibrium of specific activity of $CO_2$ appearing in urine of sheep in acidosis conditions. It was concluded that urine samples taken when an animal is in an acidosis condition are not suitable for estimating rate of energy expenditure using the carbon dioxide entry rate method.

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# INTRODUCTION

A recently developed technique for estimation of energy expenditure of free ranging animals based upon measurement of entry rate of carbon dioxide has been described by Young et al. (1969) and Corbett et al. (1971).

While the method usually worked satisfactorily for sheep and cattle at pasture, it was occasionally observed that abnormally high entry rate values occurred when urine samples were used to isolate the carbon dioxide for specific activity analysis. These abnormal values appeared to occur whenever the animal was in an acidosis condition.

The present study was made to determine the effect of acidosis on estimates of carbon dioxide entry rate based on carbon dioxide derived from various sites in the body.

In this thesis the term carbon dioxide ($CO_2$) has been used for all forms that are in freely interchanged with acid volatile $CO_2$ in blood. These forms include gaseous $CO_2$, dissolved $CO_2$, bicarbonate and carbonate. The common form of expression of these forms of $CO_2$ has been in terms of carbon (c) content.

Conversion factors for the various form of $CO_2$ compounds are presented below.

| Compound | Equivalent to $CO_2$ expressed as | | |
|---|---|---|---|
| | Carbon (mg) | $CO_2$ (mg) | $CO_2$ (ml; STP) |
| 1 ml Gaseous $CO_2$ (STP) | 0.536 | 1.964 | 1.0 |
| 1 m Eq Dissolved $CO_2$ | 12.011 | 44.011 | 22.4 |
| 1 m Eq $HCO_3^-$ | 12.011 | 44.011 | 22.4 |
| 1 m M $HCO_3^-$ | 12.011 | 44.011 | 22.4 |
| 1 m M $H_2CO_3$ | 12.011 | 44.011 | 22.4 |

# REVIEW OF LITERATURE

## A. Methods for Estimation of Energy Expenditure of Free Ranging Animals

Measurements have been made of rates of energy expenditure of animals within laboratories using various forms of direct and indirect calorimetry. These methods have been reviewed and described by Brody (1945), Douglas (1956), Wolff (1956), Kleiber (1961) and Blaxter (1967). Each of these methods require the animal to be closely confined in a chamber or restrained to prevent free movement.

Durnin and Passmore (1967) designed portable equipment for measurement of respiratory gaseous exchanges of humans and thus estimation of energy expenditure. This method has been adapted and used for field measurements on sheep by Corbett et al. (1969) and Young and Corbett (1972). The need to use a face mask which would prevent feeding activity in free ranging animals has been overcome by the development of tracheal fistulation methods for collection of respiratory gas (Flatt et al. 1958 and Young et al. 1963). The trachea canulation and the complex and bulky portable respiratory gas collecting apparatus considerably restricts the wide application of respiratory gas exchange methods for free ranging animals.

Attempts to utilize easily measured indices of energy expenditure such as heart rate (Boogens et al. 1960; Brouha 1960; Maxfield et al. 1963; Malhotra et al. 1963; Webster 1967; Brockway et al. 1969), respiratory frequences (Durnin et al. 1955; Ford et al. 1959; Malhotra et al. 1962) and body temperatures (Berggren et al. 1950) have been often suggested but to date have not proved to be satisfactory for use on field animals.

Lifson et al. (1955), McClintock et al. (1957a & b, 1968a & b), Lee et al. (1960), Lifson et al. (1961), LeFebvre (1964) and Lifson et al. (1966) have developed and tested a method for estimating rates of energy expenditure of small animals from the apparent turnover rate of hydrogen and oxygen in body water. These turnover

rates are estimated by the use of deuterium and oxygen-18 double labelled water. The method has been used on mice, rats, and pigeons and seems suitable for application for field conditions. The major limitation of the method is the cost of the large amount of oxygen-18 needed to establish sufficient tracer in the animal for accurate measurement.

Young et al. (1969) and Corbett et al. (1971) reported a method for estimation of the rate of energy expenditure of unrestrained ruminants from measurements of the entry rate of $CO_2$ in the body. With this method, $^{14}C$ labelled sodium bicarbonate is infused at a constant rate into the animal to establish an equilibrium between the labelled carbon dioxide and the body pools of unlabelled carbon dioxide. The rational of the method developed by Young and co-workers is based upon the concept that the production and turnover of $CO_2$ in the body is directly dependent on the rate of energy expenditure of the animal. This method is similar to the doubly labelled water method of Lifson and co-workers but does not need the large amount of label to establish measurable equilibriums in the body and is considerably less costly.

## B. Detail Consideration of the Carbon Dioxide Entry Rate Method

Komberg et al. (1952) and Steele (1955) derived a three-compartment kinetic model for the retention of $CO_2$ in the cat. The model was considered to contain a central carbon dioxide compartment which represented the circulating blood,and two peripheral compartments represented by (a) the solid carbonate in bone with its relatively slow turnover rate and (b) the $CO_2$ in soft tissues with a more rapid carbon dioxide turnover rate. It was considered that the $CO_2$ was produced principally from the cell metabolism of the two peripheral compartments and transfered into the central compartment of circulating blood. The carbon dioxide entry rate in the central blood compartment of the body can be considered as the $CO_2$ turnover rate in that compartment which is equivalent to the $CO_2$

elimination rate and proportional to the metabolic rate (oxidation rate) within the animal.

Oxidative metabolism has been extensively studied in ruminants using $^{14}C$ labelled metabolites. The metabolites have been administered to the animals by either single injection (Kleiber et al. 1952; Black et al. 1957 and Annison et al. 1961) or by continous infusion (Essig et al. 1961 and Sabine et al. 1964). Likewise, $CO_2$ entry rate values may be determined by administration of $^{14}C$ labelled carbon dioxide either as a single injection or continuous infusion and observing the changes in specific activity (SA) of $CO_2$ in body fluids. A short term difference in the entry rate (production rate) and the rate of elimination of carbon dioxide from the body could arise if a change occurs in the magnitude of the carbon dioxide storage in the various body pools with which metabolically produced carbon dioxide mixes to a variable extent before it is eliminated from the body. Morris and Simpson-Morgan (1963) considered that a more precise estimate of entry rate was obtained by means of the continuous infusion technique than use of the single injection method of administering labelled carbon dioxide. Young (1968) pointed out some of the difficulties of interpretation of results of single injection experiments when a steady state is not achieved. Young concluded that estimated entry rates obtained refer only to the period of measurement which can not be, for a single injection experiment, more than a few hours. Estimates of energy expenditure made over periods of 24 hours or longer are generally more useful in studies on free ranging animals. For these reasons studies involving the estimation of entry rate of carbon dioxide of free ranging animals have used constant rates of infusion of $^{14}C$ labelled carbon dioxide. Infusions must be continued for sufficient time to allow complete mixing of the infusate with the body pool of carbon dioxide. Once equilibrium is reached, the specific activity of the carbon dioxide will depend upon the entry rate of carbon dioxide and thus the rate of energy expenditure of

of the animal.

At equilibrium, the carbon dioxide entry rate (mgC/min) is equal to the $^{14}C$ infusion rate (n ci/min) divided by the specific activity of the carbon dioxide in the sample (n ci/mgC).

The entry rate calculated by the above equation may differ somewhat from the actual rate of production of $CO_2$ within the body if all $CO_2$ produced and infused does not enter the compartment that is sampled (Young, 1968).

The following are examples of possible interference to carbon dioxide entry rate measurements, and could cause errors or biases in estimates.

a. Methane and urea formation

In studies of rumen function, Ash et al. (1963) found that the epithelial tissue of the reticulum is permeable to bicarbonate and carbon dioxide. $CO_2$ that enters the rumen can readily react in several fixation processes such as the formation of methane and subsequently be lost in eructated gases (Carroll et al. 1955; Williams et al. 1963 and Hungate 1966). Urea synthesis occurs in the liver where $CO_2$ and ammonia are condensed via the urea cycle (Harper 1969). Rust et al. (1963) found radioactivity in urea of urine from rats following injection of $NaH^{14}CO_3$.

b. Carbon dioxide fixation in tissues

Carbon dioxide is incorporated into body metabolites as a result of various metabolic processes in living tissues (Wood et al. 1945; Ochoa et al. 1948; Skipper et al. 1949; Shreeve 1952; Komberg et al. 1952; Utter 1959; Rust et al. 1963; Wood et al. 1965; Witternan et al. 1967 and Milligan 1970). Krebs (1951) found an average 1.37% of $^{14}C$ was fixed into mouse tissue after infusion of the $NaH^{14}CO_3$. Wittenan (1967) analyzed the fixation of $^{14}C$ from $CO_2$ in swine tissues. The results of these studies are summarized in Table 1.

Table 1 Distribution of $^{14}C$ in tissue after injection of 750 μci of $Na_2{}^{14}CO_3$ into an 8 kg pig. Calculated from Wittenan (1967).

| Time after injection | % of injected label in tissue | | | | | |
|---|---|---|---|---|---|---|
| | Heart | Fat | Kidney | Liver | Skin | Muscle |
| 9 days | 0.75 | 0.42 | 0.57 | 1.05 | 0.31 | 0.52 |
| 21 days | 0.38 | 0.26 | 0.55 | 0.25 | 0.22 | 0.45 |

c. Dilution of labelled carbon dioxide with locally produced carbon dioxide

Robinson et al. (1957) found that all tissues of the body did not equilibrate with $CO_2$ at the same rate. Furthermore, Coxon (1959) found the movement labelled $CO_2$ differed in various tissues following administration of $^{14}C$ glucose and he further showed that $CO_2$ was produced from glucose oxidation at considerably different rates in different organs of the body.

Young (1970) suggested that although an equilibrium specific activity of $CO_2$ may be reached during constant infusion of $^{14}C$ labelled $CO_2$ the specific activities of carbon dioxide in various body fluids is not necessarily the same. Any difference may reflect the relative local dilution of the labelled $CO_2$ by unlabelled $CO_2$ which is produced from regional metabolism. Also carbon dioxide produced from metabolism in the lungs, kidney and rumen may be eliminated from the body without entering into the central blood $CO_2$ compartment.

d. The pH of body fluids

The pH of blood and body fluids are greatly influenced by the bicarbonate to carbonic acid ratio in the blood compartment. Thus variations in $CO_2$ elimination rate from the body arising from pH changes may influence the entry rate values derived from samples taken from various sites in the body.

## C. Detail Consideration of pH on $CO_2$ Kinetics

### a. Acid-base balance

The respiratory system has the major role in regulation of acid-base balance and the kidney has a compensatory role. Pitts (1968) estimated that in the human lung about 13,000 m Eq/24h of carbonic acid was excretion as gaseous $CO_2$ and the kidneys excrete 40 to 80 m Eq/24 h of non-volatile acid as titratable acid ($H_2PO_4^-$) and ammonium ion ($NH_4^-$) which were accomplished in part by the re-absorption of bicarbonate.

The bicarbonate-carbonic acid buffer system in the blood has a key role in maintenance of the pH. At normal pH of blood and body fluids the molar ratio of bicarbonate to carbonic acid is 20:1 (Pitts 1968, Ganong 1969). The carbonic acid concentration is regulated by the respiratory system and the bicarbonate ion concentration is regulated by the kidney. This relationship of regulation is illustrated in Figure 1 which is based on studies made by Christensen (1963) on humans.

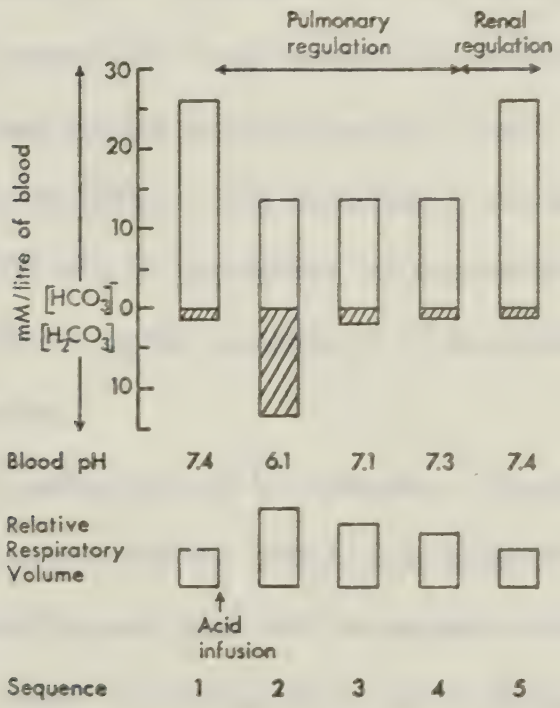


Figure 1. Stepwise pulmonary and kidney response to acid invasion. From Christensen (1963).

Christensen's interpretation is summarized in Fig. 1 and shows that with the normal pH of blood (7.4) the ratio between $HCO_3^-$ and $H_2CO_3$ is 26:1.3 (mM/L), sequence 1. If acid is injected to change the ratio to 13.7:13.7 (2) then by the Henderson-Hasselbach equation the pH should be 6.1. Actually the incoming acid strongly stimulates the respiratory rate and volume so the $H_2CO_3$ is quickly dissociated to $CO_2$ and $H_2O$ and the $CO_2$ is swept out by pulmonary activity so that the pH never in fact falls as low as 6.1. The animal continues with an accelerate respiration and brings the $H_2CO_3$ concentration to a normal level (3 to 4). At this stage the pH is still low until the renal activity is able to return the bicarbonate level to a normal value (5).

b. Renal tubular reabsorption of bicarbonate

In the studies on man Pitts and Lotspeich (1946) showed that when plasma bicarbonate concentration was reduced through the injection of $NH_4Cl$, all of the bicarbonate filtered through the glomeruli was reabsorbed. This reabsorption continued as long as the concentration of bicarbonate in the plasma remained below 26 to 28 mM/L. This value is termed the "renal bicarbonate threshold". With higher blood bicarbonate levels a minimal amount of bicarbonate (2.8 mM/100 ml. of glomerular filtrate) is reabsorbed by the kidneys, the remainder is excreted into urine. Pitts (1968) reported that about 5100 mEq of bicarbonate ion are reabsorbed each day in man from the glomerular filtrate by the secretion of an equivalent number of hydrogen ions into the tubular urine.

Renal reabsorption of bicarbonate in the dog was similarly controlled (Pitts et al. 1949) except that the "renal bicarbonate threshold" value was lower (24 to 26 mM/L) than for man, and with the excessive blood bicarbonate the reabsorption rate slightly less (2.6 m-mole/100 ml of the glomerular filtrate). Pitts and co-workers also found a curvilinar relationship between urine bicarbonate concentration and urine pH. This relationship was confirmed in sheep (Scott 1969 and Nave et al.

1969), calves (Scott et al. 1971a), red deer (Scott 1971b) and pig (Scott, 1971c). Apparently the renal control of bicarbonate excretion in ruminants (sheep, cattle and deer) is similar to that in man, dog and pig.

c. Mechanism of renal reabsorption of bicarbonate

The renal tubular secretion of hydrogen ions in exchange for sodium ion is the major mechanism of reabsorption of bicarbonate (Pitts 1945a; Pitts et al. 1945b; Berliner 1952; Brazeau et al. 1955; Relman et al. 1953; Rector et al. 1960; Rector et al. 1965). The secreted hydrogen ions apparently react with filtered $HCO_3^-$ to form $H_2CO_3$ which then dissociated to $CO_2$ and $H_2O$, the $CO_2$ being reabsorbed. Rector et al. (1965) observed that the pH of urine in the distal tubules of rats averaged 0.85 units less, i.e. more acid, than the filtrate. They concluded that ion exchange and bicarbonate reabsorption occurs in both the proximal and distal tubules.

# EXPERIMENTAL PROCEDURES

## I Materials and Methods

### A Animals

#### a. Sheep

Four two year old Lincoln ewes were used in the study (Table 2).

Table 2 Experimental sheep

| Sheep No. | Wt. kg | Experiment | |
|---|---|---|---|
| 308 | 65 | A | |
| 48 | 78 | A | |
| 8224 | 62 | A | B |
| 116 | 81 | A | B |

The sheep were kept in a heated laboratory in individual crates. Early in the study, all the sheep were prepared with carotid loops and rumen fistula. The sheep received 500 grams of alfalfa pellets twice daily (8.30 and 16.30 hr) for at least 6 weeks before trials were made. Cobalt-iodized salt blocks and water were available ad libitum. All feed, water and salt blocks were withheld during the infusion trials which commenced on each occasion shortly after the sheep had consumed their morning ration.

#### b. Cattle

Two female cattle were used for the infusion trials within the laboratory; a year-old Hereford heifer (No. 3, weight 290 kg) which was previously prepared with a carotid loop, and a two-year-old Hereford cow (No. 32, weight 402 kg) which was previously prepared with a rumen fistula. Other than during the infusion

period these cattle were kept in a small holding pasture adjacent to the laboratory. The following cows and heifers were used in the field studies.

Table 3 Cattle used for field studies

| | | | |
|---|---|---|---|
| No. 35 | 342 kg | 24 months | milking with calf |
| No. 41 | 351 kg | " | " |
| No. 144 | 452 kg | " | " |
| No. 36 | 382 kg | " | dry without calf |
| No. 378 | 342 kg | 14 months | young growing heifer |
| No. 108 | 341 kg | " | " |
| No. 165 | 296 kg | " | " |

B. Preparation of Animals

a. Surgery preparation

A carotid loop and rumen fistula were established in each of the sheep prior to their use (photo. 1). The carotid loops were established by the method described by Butler (1962) and the rumen fistula were established by the method described by Hecker (1969).

b. Training of animals

All animals which were used in the studies had been used in previous laboratory experiments and were somewhat accustomed to some of the experimental procedures. The animals which were used in the laboratory studies were subjected to training to make them thoroughly accustomed to the face mask which they were required to wear continuously for several hours at a time.

The cattle which were used in field studies were accustomed to accept the infusion and sampling equipment which was mounted on their backs. This was achieved by mounting firstly the harness then at intervals additional weights and pieces of equipment were added. This training period with the cattle usually

Photo 1 Sheep with carotid loop (above) and rumen fistula (below).

occupied approximately two weeks.

c. Catheterization

Catheters were inserted into the jugular vein, carotid artery and urinary bladder of the animal. Catherizations were made either the night prior to the trial or approximately 1 hr before the start of the infusion period.

(1) Jugular vein

An intramedic polythene catheter (PE-90/S36 or PE-190/S36, Clay - Adams INC, New York, USA) was inserted into either of the external jugular vein via a two inch, 13 gauge, bleeding needle. The catheter was threaded down to the right ventricle. Location of the end of the catheter in the right ventricle was detected by pulsation of a small air bubble introduced into the saline-filled catheter. Novocain (Winthrop Laboratories, Aurora, Ontario, Canada) was injected subcutaneously whenever skin sutures were required to secure the catheters in position. The catheters were filled with heparinized saline (200 USP units of heparine per ml of physiological saline) and close off with a three way tap (MS 10, Stevens Co., Calgary, Canada) which was connected to the catheter by an 18 or 16 gauge needle. The three way tap was then tied to the collar about the animal's neck.

(2) Carotid artery

An intramedic polythene catheter was inserted into the exteriorized carotid artery by a procedure similar to that of the jugular vein. However, the catheter was inserted only 10 to 12 cm into the artery.

(3) Urinary bladder

A Bardex catheter (Stevens Co.) was inserted into the urinary bladder. Catheter sizes of 14 FR. and 22 FR. were used for sheep and cattle, respectively.

(4) Intraperitoneal

A intramedic polythene catheter (PE205 or PE320, Clay - Adams)was inserted through the abdominal wall on the right flank of the sheep or cattle. The procedure has previously been described in a thesis by Young (1968). A brief description of this method is given here.

The hair or wool was closely clipped from the site for insertion of the catheter and a local anaesthetic (Novocain) was administered. A two inch, 13 gauge, bleeding needle was then inserted through the abdominal wall and a 12 cm length of guide wire with a 11.5 cm length of catheter was passed through the needle and threaded about 10 cm into the animal. The wire was then removed by passing the catheter over it. Catheter was sutured in position to avoid the possibility of the catheter either entering or pulling from the abdominal cavity. A long length of the same size catheter, with a short section of 22 gauge needle as a joiner, was used to connect the catheter to the infusion pump.

C. Administration of Isotope

a. Preparation of infusion solution

Carbon-14 sodium bicarbonate ($NaH^{14}CO_3$) were obtained from the Radio-Chemical Center, Amersham/Searle Co. The $NaH^{14}CO_3$ was diluted to the required specific activity for infusion with isotonic non-labelled sodium bicarbonate (1.4% W/V of $NaHCO_3$)

b. Pump and infusion rate

Lambda pumps (Model 1300, Harvard Apparatus Co. Inc., Dover, Mass., USA) were found suitable for continuous infusion of $NaH^{14}CO_3$ and had a coefficient of variation of less than $\pm$1% in their pumped volume (Young 1968). During laboratory studies the Lambda pumps were activated by a pump driver (Model 1305). For field studies the pumps were activated by re-chargeable batteries and pulse generators

(Model 1301).

The $NaH^{14}CO_3$ was infused at rates of approximately 30 and 200 n ci per minute for sheep and cattle, respectively, in a volume of 0.1 to 0.2 ml per minute of $NaHCO_3$ solution. The exact infusion rate (n ci/min) was calculated from the pumping rate (ml/min) and the specific activity strength of the infusion solution (n ci/ml).

D. Administration of Acid

To experimentally change the acid level in the body 1 N HCl acid was infused at a constant rate into the rumen of the sheep. This infusion was made via the rumen fistula. The plug of the rumen was replaced by a special plug designed with an infusion catheter through it. The special plug sealed the fistula to prevent rumen fluid or gas escaping during the period of the infusion but allowed the infusion catheter to be moved to new sites in the rumen every 15 minutes to aid in the mixing of the acid with a rumen contents.

The acid was pumped into the rumen by means of a variable speed infusion pump (series 950V, Harvard Apparatus Co.). The infusion rate of acid was approximately 20 mEq per minute.

The acid infusion system was set up prior to the start of each trial but not activated until one hour after the estimated time for the infused $^{14}CO_2$ to reach plateau equilibrium with the carbon dioxide in the central blood compartment.

E. Sampling Procedures

a. Blood

Arterial and venous blood samples were taken from the carotid artery and right ventricle via the established catheters. The blood was drawn into disposable plastic syringes containing a drop of heparin (Riker Pharmaceutical Co., Cooksville,

Ontario, Canada). The inside of the barrel of each sample syringe was lightly greased to prevent entry of air which could dissolve in the blood sample and cause an incorrect measurement of blood gas content. After taking the blood samples the syringes were immediately capped and placed in an ice bath until the samples were analyzed. Immediately after the samples were taken the catheters were flushed with heparinized saline.

b. Urine

Urine samples were collected via the established catheter into a 50 ml measuring cylinder. The collection periods were 15 or 30 minutes. After each collection, the volume of urine was measured and the sample was taken and placed in a sealed vial and stored at 4°C until analyzed.

c. Gaseous $CO_2$

Expired $CO_2$ from the animal was collected via the respiratory gas apparatus (see below). A continuous sample of expired gas (10 ml per minute) was bubbled into 5 ml of 1N $CO_2$-free NaOH. After each 15 or 30 minutes the container of NaOH was exchanged with a new container. After the completion of the bubbling period 2 ml of 5% (W/V) $NH_4Cl$ and 1 ml of 20% (W/V) $BaCl_2.2H_2O$ were added to precipitate the $CO_2$ as $BaCO_3$. The container was then sealed and stored at 4°C until radioassay.

d. Milk

Milk samples were collected during one trial only. These were taken from cow No. 32 which was used during a laboratory trial. Ten ml of milk was taken from the right hind quarter of the udder every 30 minutes. All remaining milk was removed from the quarter immediately after the sample was taken. The sample was sealed and stored at 4°C until analysis was made.

e. Rumen fluid

Rumen samples were collected for radioassay from only cow No. 32 used in a laboratory trial. Samples (10 ml) were taken via the rumen fistula, they were immediately sealed and stored at 4°C until radioassay.

F. Respiratory Gaseous Exchange

Estimates of rates of energy expenditure were obtained from measurements of rates of oxygen consumption and carbon dioxide production. Continuous measurements of respiratory gaseous exchanged were made using an open circuit apparatus similar to that described by Webster and Hicks (1968).

During each laboratory experiment a mask was placed on the face of the sheep or cattle and ventilated at approximately 60 litres per minute with atmospheric air. A precise measurement of the ventilation rate was obtained from a dry-gas meter in the respiratory gas analyzer. The oxygen and carbon dioxide contents of the air drawn from the animal were measured continuously using a Beckman F-3 oxygen analyzer and an IR-215 carbon dioxide analyzer, respectively (Beckman Instruments, Inc., Fullerton, California, USA). Oxygen consumption and carbon dioxide production were calculated by multiplying the ventilation rate (liters/min at STP - dry) by the decrement of oxygen or the increment of carbon dioxide observed between the air entering and leaving the ventilated mask, respectively.

The rate of energy expenditure (kcal/min) was calculated from the oxygen consumption (liter/min at STP - dry) and the carbon dioxide production (liters/min at STP - dry) using the formula of Brouwer (1965) as follows: EE(kcal/min = $O_2$ (liters/min) x 3.866 + $CO_2$ (liters/min) x 1.2.

A small amount of $CO_2$ was collected from the open circuit respiratory apparatus for radioassay. The method of collection and the radioassay method are described elsewhere in the Experimental Procedures.

G. Gas and Acid-Base Analysis in Body Fluids

The Radiometer Blood Micro-system (BMS3, Radiometer, Copenhagen, Denmark) with the Model G298A pH and model E5036 $pCO_2$ electrodes in a thermostatically controlled bath (39°C) were used to analyze arterial blood, venous blood and urine for pH and $pCO_2$,

The concentration of bicarbonate (Eq/liter) and the total concentration of carbon dioxide (Eq/liter) were calculated from the Henderson-Hasselbach equation employing a pKa of 6.1 and a solubility coefficiant (S) of 0.03 m Eq/liter/mm Hg.

Thus:
$$pH = pKa + \log \frac{HCO_3^-}{S \cdot pCO_2}$$

$$pH = pKa + \log \left(\frac{\text{Total } CO_2}{S \cdot pCO_2} - 1\right)$$

H. Radioassay of Carbon Dioxide

Determination of specific activity of $CO_2$ in samples of blood, urine, milk, rumen fluid and expired gas were made by procedures similar to those described by Annison and White (1961), Leng and Leonord (1965) and Young (1968).

Ten to 30 ml samples of blood, urine, milk, or rumen fluid were placed into a 50 ml of Titeseal vial (Chemical Rubber Co., Cleveland, USA). A small glass tube (No. 9820, pyrex) containing approximately 1 ml of 1N $CO_2$-free NaOH was then placed within the larger vial and the cap of the vial immediately resealed. Approximately 1 ml of 1 N $H_2SO_4$ containing 1% (W/V) of $CuSO_4$ was then injected into the sample through the margin of the cap. Following this acidification gaseous $CO_2$ transferred from the sample to the NaOH solution. Forty-five hours was allowed for the transfer of the $CO_2$ from the sample to the NaOH solution. The small glass tube was then removed from the vial and one ml of 5% (W/V) $NH_4Cl$ and 0.5 ml of 20% (W/V) $BaCl \cdot 2H_2O$ were added to precipitate any carbonate as the barium salt.

The $BaCO_3$ from the blood, urine, milk and rumen fluid samples and the samples collected from the respiratory gas were filtered with Genuine Whatman filter papers (No. 50, W and R Co., Boston, USA) on a Buchner funnel and washed with distilled water and acetone. The filter funnel could be dissociated such that the filter paper and precipitate could be easily removed. The precipitate of barium carbonate containing $^{14}C$ was placed on a rotating lamp drier, (Model Spp-69 Atomic Accessories Inc., Valley Stream, New York, USA) for half an hour and then ground to a fine powder with a spatula. A weighed amount usually 20 to 30 mg of the ground $BaCO_3$ was placed in a scintillation vial (Scientific and Process Instruments Div. Beckman, California, USA). The $BaCO_3$ was then suspended in 10 ml of scintillation fluid containing 3.4% Carbo-sil, 0.01% 5-phenol-oxazole (popop) and 0.4% 2.5-diphenyloxozole (ppo) in xylene. The amount of radioactivity in the samples was then measured in a liquid scientillation counter (Model DS-5, Nuclear Chicago Corp. Des Plains, Illinois). The channels ratio method of Hendler (1964), Horrocks (1968) and Turner (1969) were used to correct for extraneous quenching.

Preliminary tests showed that approximately 45 hours was required for complete transfer of labelled $CO_2$ from the samples to the hydroxide solution. These tests showed 85% of label has been transferred after 5 hours and a progressive increase in recovery with the maximum being reached at about 45 hours.

The function of the copper sulfate addition to the acid was to prevent microbial fermentation which could produce unlabelled carbon dioxide and upset the specific activity measurements.

Preliminary tests showed that when $CuSO_4$ was not added with the acid, the specific activity of the isolated $CO_2$ was about 5% lower than when $CuSO_4$ was added (Table 4).

Table 4 Preliminary tests of time requirement for complete transfer of label from sample to 1 N of NaOH.

| Time after acidification (hours) | Specific Activity n ci/carbon (mg) | |
|---|---|---|
| | add $CuSO_4$ | without $CuSO_4$ |
| 5 | 0.1643 | 0.1603 |
| 21 | 0.1805 | 0.1761 |
| 27 | 0.1868 | 0.1837 |
| 45 | 0.2112 | 0.2063 |
| 51 | 0.2188 | 0.1983 |
| 69 | 0.2181 | 0.2011 |
| average | 0.1966 | 0.1876 |
| % | 100% | 95% |

The specific activities of the infusion solutions were measured by the above procedure after dilution with standard unlabelled bicarbonate solution.

## I. Statistical Methods

Statistical analysis of data were made by methods described by the Steel and Tory (1958).

## II. EXPERIMENTS

### A. Experiment A Infusion of $NaH^{14}CO_3$

$NaH^{14}CO_3$ were infused into the 4 sheep, one heifer (No. 3) and one cow (No. 32) for 390 to 420 min. During the infusion trials the animals were confined indoors in metabolism crates (sheep) or stranchions (cattle) and the respiratory gas exchange equipment was attached to the animal (photo 2). The samples of arterial blood, venous blood, urine, expired gas, rumen fluid and milk were taken immediately

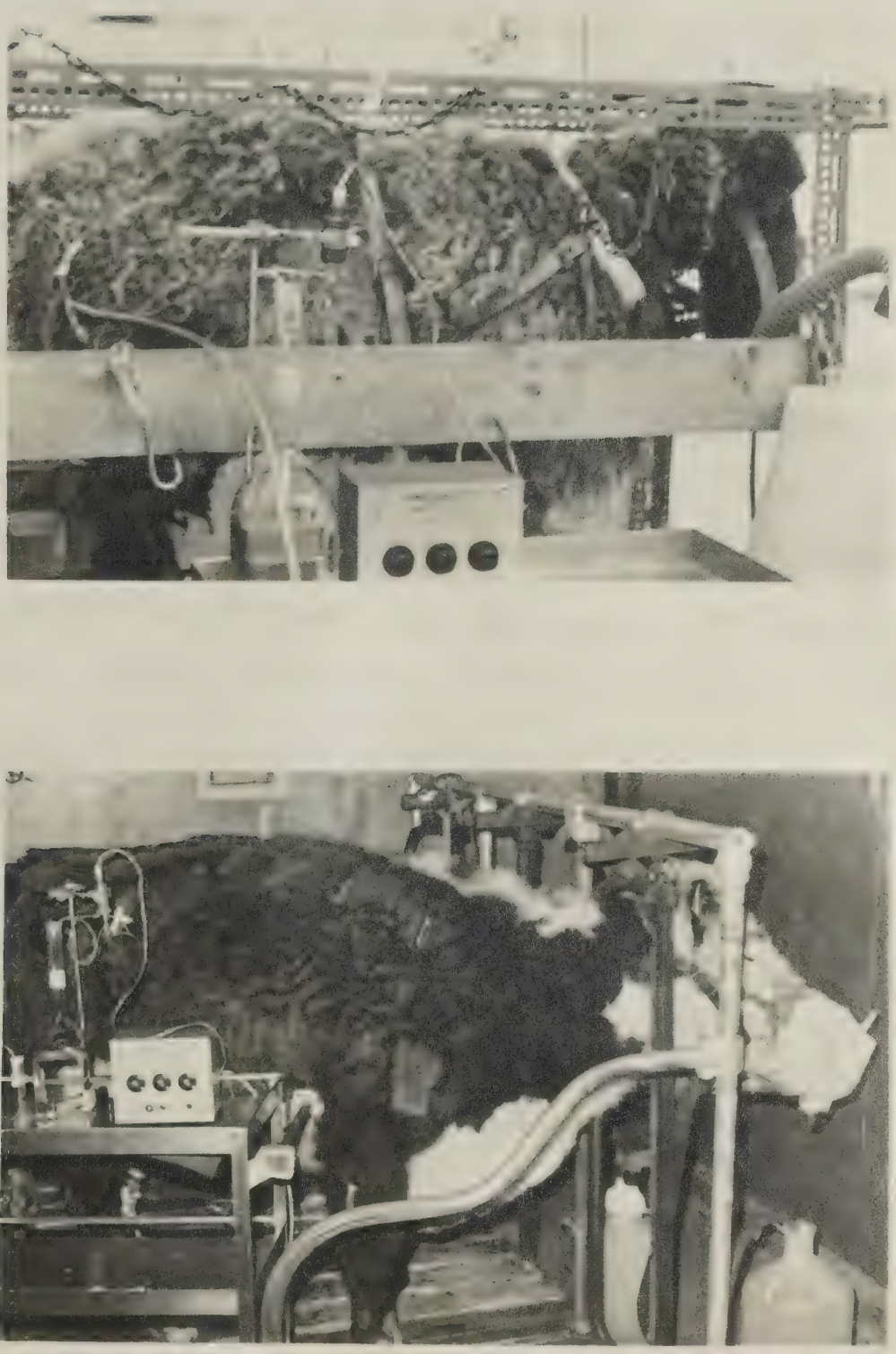

Photo 2 Sheep and cattle with respiratory mask and isotope infusion equipment.

prior to the infusion and at 15 or 30 minute intervals thereafter for radioassay. Except on some occasions samples were not taken prior to the anticipated plateau equilibrium. No arterial blood was taken from cow 32 and samples of rumen fluid and milk were taken from this cow only.

B. Experiment B Infusion of $NaH^{14}CO_3$ plus Acid Infusion

Isotope infusion and respiratory gas exchange measurement were made in the same manner as for Experiment A but only two (No. 116 and No. 8224) of the four sheep were used. The infusion periods were extended to 480 or 585 min. Acid was infused into rumen after the fourth hour of isotope infusion. Samples of arterial blood, venous blood, urine and expired gas were taken for radioassay. As well samples of arterial blood, venous blood and urine were taken for measurement of pH. The $pCO_2$ contents of the urine samples were also determined.

C. Experiment C Measurement on Field Animals

$NaH^{14}CO_3$ was infused continuously for approximately 29 hours into seven heifers and cows (Table 3). After 5 hours of infusion continuous sampling of urine was commenced. Samples of jugular blood were taken transiently at the fifth and 29th hour of infusion. The samples of urine and blood were analyzed for SA of $CO_2$ only.

The equipment for infusion and sampling has been described by Young (1970). A brief description of this method and equipment with additional information not contained in Young's publication is as follows:

The battery powered Lambda pumps (Harvard Instrument Co.) were used for both the infusion and sampling systems (photo 3). Two transfer packs (Fenwall Laboratories Morton Grove, Illinois, USA) of 150 ml capacity were sufficient for periods of up to 36 hr of infusion and sample collection.

The infusion and sampling systems were housed independ ntly in wooden boxes and mounted on a carrying belt over the shoulders of the animals. As well

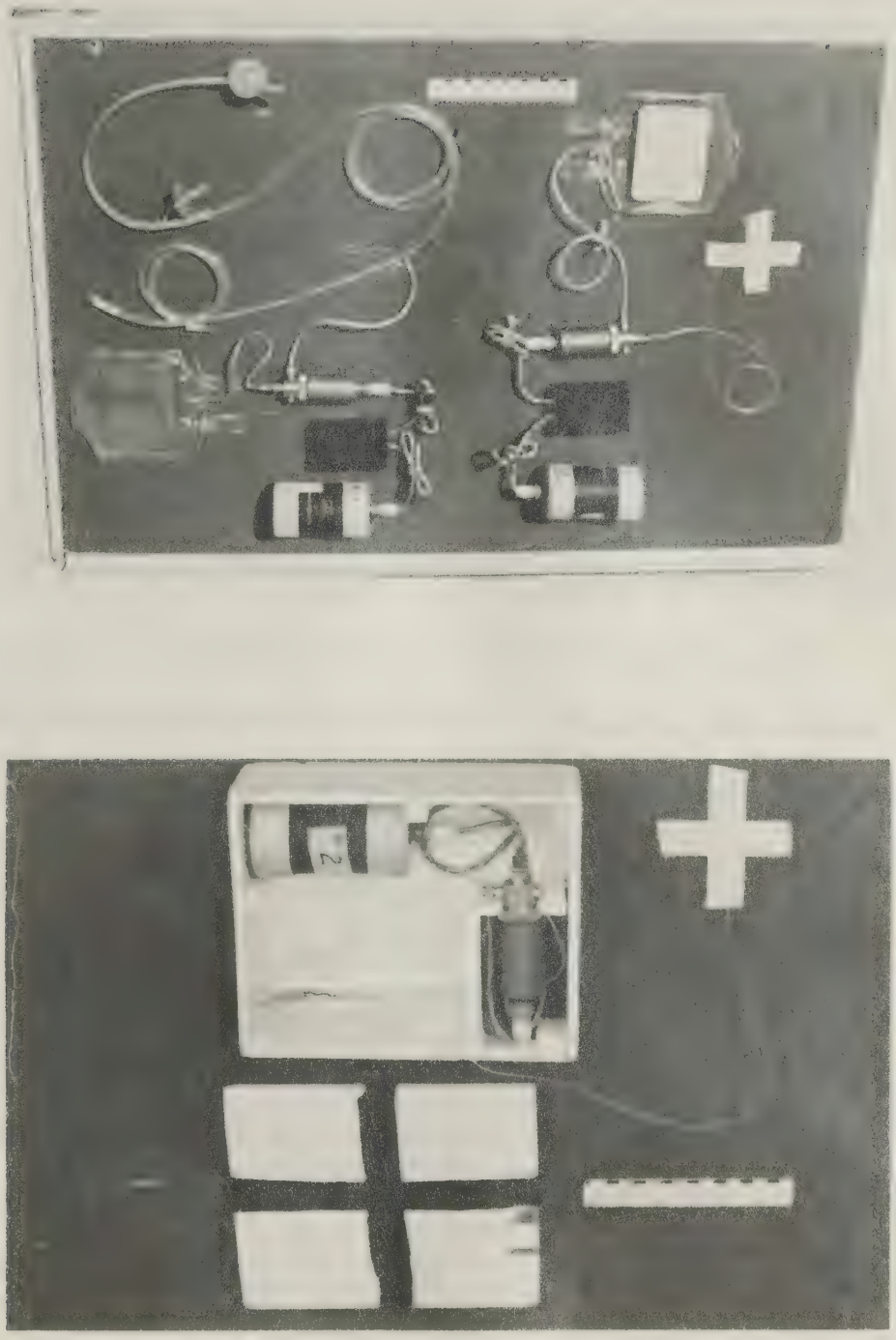

Photo 3 Sampling system (left above) and Infusion system (right above) and its housing box (below) used for field measurement of carbon dioxide entry rate.

as the girth strap an extra supporting strap passed around the shoulders of the animals and another strap (crop) to the base of the tail (photo 4).

The infusion of $NaH^{14}CO_3$ were made intraperitoneally through the right flank 15 cm behind the last rib and 20 cm down from the vertebrae. The infusion catheter was sutured to the skin and covered with adhesive tape to hold it in place. The infusion method is described above.

The urine sampling system consisted of a Bardex Catheter (22 FR, The Steven's company) which was inserted into the urinary bladder. The urinary catheter was then connected to a flexible plastic tube which was tied to the strap extending from the base of the tail to the carrying pack. The tube was then divided into two, one branch passed to the sampling pump which withdrew and deposit the sample (approximately 0.1 ml/min) in the collecting container which was sealed but flexible thus allowing the accumulation of sample without exposure to the atmospheric air. The other branch of the urine line extend under the animal and allowed the urine, which was not drawn off by the sampling pump, to fall into the ground. It was necessary to have a rubber valve at the end of the latter duct to prevent the back flow of urine. The urine sampling system was not activated until the infusion system had been operating for at least five hours to allow the body pool of $CO_2$ to reach an equilibrium with the infusate.

Photo 4 The Sampling system and infusion system in harness (above) and attached to a cow in the field (below).

# RESULTS

## A Experiment A $NaH^{14}CO_3$ Infusion

### a. Time to reach plateau

The specific activity of $CO_2$ in venous blood, arterial blood, urine and expired gas increased rapidly with $NaH^{14}CO_3$ infusion and reached plateau levels with only a slight variation after the infusion had been in progress for about 3 hours in sheep and 4 hours in cattle. The typical result of both sheep and cattle are shown in Fig. 2. Detailed results of each trial in experiment A are contained in Appendix A.

### b. Local dilution

The specific activity of $CO_2$ in arterial blood, venous blood, urine, expired gas, rumen fluid, and milk had different plateau levels. The specific activity of $CO_2$ in rumen fluid and milk had relatively large variations and were significantly lower ($p < 0.01$) than that of arterial blood, venous blood, urine and expired gas. Relative values are summarized in Table 5. Appendix A contains individual observations.

Table 5 Relative value of specific activity of $CO_2$ in body fluids at plateau

| Animal | Relative to arterial blood (%) | | | Relative to venous blood (%) | | | | |
|---|---|---|---|---|---|---|---|---|
| | Venous Blood | Urine | Expired Gas | Arterial Blood | Urine | Expired Gas | Rumen Fluid | Milk |
| Sheep No. 48 | 95.3 | 100.6 | 75.9 | 106.8 | 105.5 | 79.6 | - | - |
| Sheep No. 116 | 87.6 | 106.2 | 81.9 | 114.0 | 121.2 | 94.2 | - | - |
| Sheep No. 224 | 96.2 | 149.3 | 89.5 | 103.8 | 155.1 | 93.0 | - | - |
| Sheep No. 308 | 84.1 | 127.2 | 85.2 | 118.8 | 150.4 | 101.3 | - | - |
| Sheep No. 48 | 86.8 | 132.7 | 92.7 | 115.1 | 152.8 | 106.8 | - | - |
| Cattle No. 3 | 92.0 | 116.0 | 90.2 | 108.6 | 126.1 | 98.0 | - | - |
| Cattle No. 32 | - | - | - | - | 118.5 | 85.9 | 59.4 | 45.6 |
| mean | 90.1 | 122.0 | 85.9 | 110.8 | 132.8 | 94.1 | 59.4 | 45.6 |

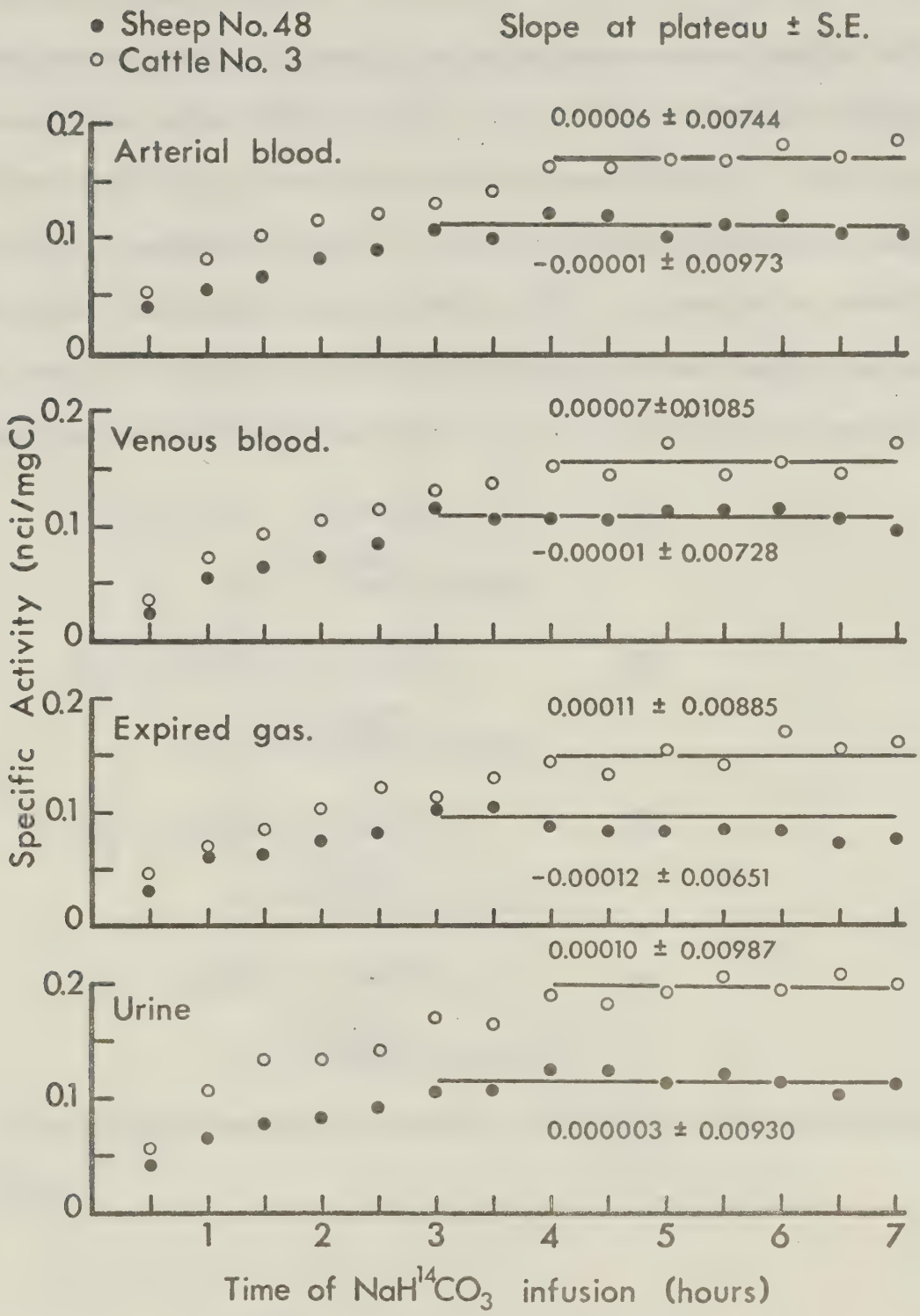


Figure 2. Specific activity of $CO_2$ in the body fluids during intraperitoneal infusion of $NaH^{14}CO_3$. Infusion rate were 25.5 n ci/ min and 171.3 n ci/min in sheep and cattle, respectively. For details see Appendix A 1 and A 6.

c. Relationships between entry rate and energy expenditure

Rate of energy expenditure (EE; kcal/min) of four ewes and two cows, estimated from the respiratory gaseous exchanges,were regressed against carbon dioxide entry rate values (ER;mgC/min). The entry rate values were estimated from specific activity of $CO_2$ in arterial blood (a), venous blood (v), urine (u) and expired gas (g), respectively (Fig. 3). The following are the regression equations derived, where the standard error of estimate (S.E.) and correlation co-efficient (r) were based on 58 observations of venous blood, urine and expired gas and 46 observations on arterial blood (Appendix A 2, A 3, A 4, A 5, A 6, and A 7).

$$EE_v = 0.1401 + 0.0045\ ER_v \qquad (1)$$
$$r = 0.9856$$
$$SE = 0.3803 \text{ kcal/min}$$

$$EE_u = 0.4793 + 0.0052\ ER_u \qquad (2)$$
$$r = 0.9890$$
$$SE = 0.3324 \text{ kcal/min}$$

$$EE_g = 0.4569 + 0.0037\ ER_g \qquad (3)$$
$$r = 0.9752$$
$$SE = 0.4570 \text{ kcal/min}$$

$$EE_a = 0.4369 + 0.0043\ ER_a \qquad (4)$$
$$r = 0.9721$$
$$SE = 0.2883 \text{ kcal/min}$$

The individual regression equation (1), (2), (3) or (4) were all significant ($p < 0.01$).

## B Experiment B $NaH^{14}CO_3$ Infusion plus Acid Infusion

Fig. 4 illustrates the effect of acid infusion on blood pH, urine pH, elimination rate of $CO_2$ via urine and expired gas, and on the specific activity of $CO_2$ in venous blood, arterial blood, expired gas and urine (see Appendix B for all original results from experiment B).

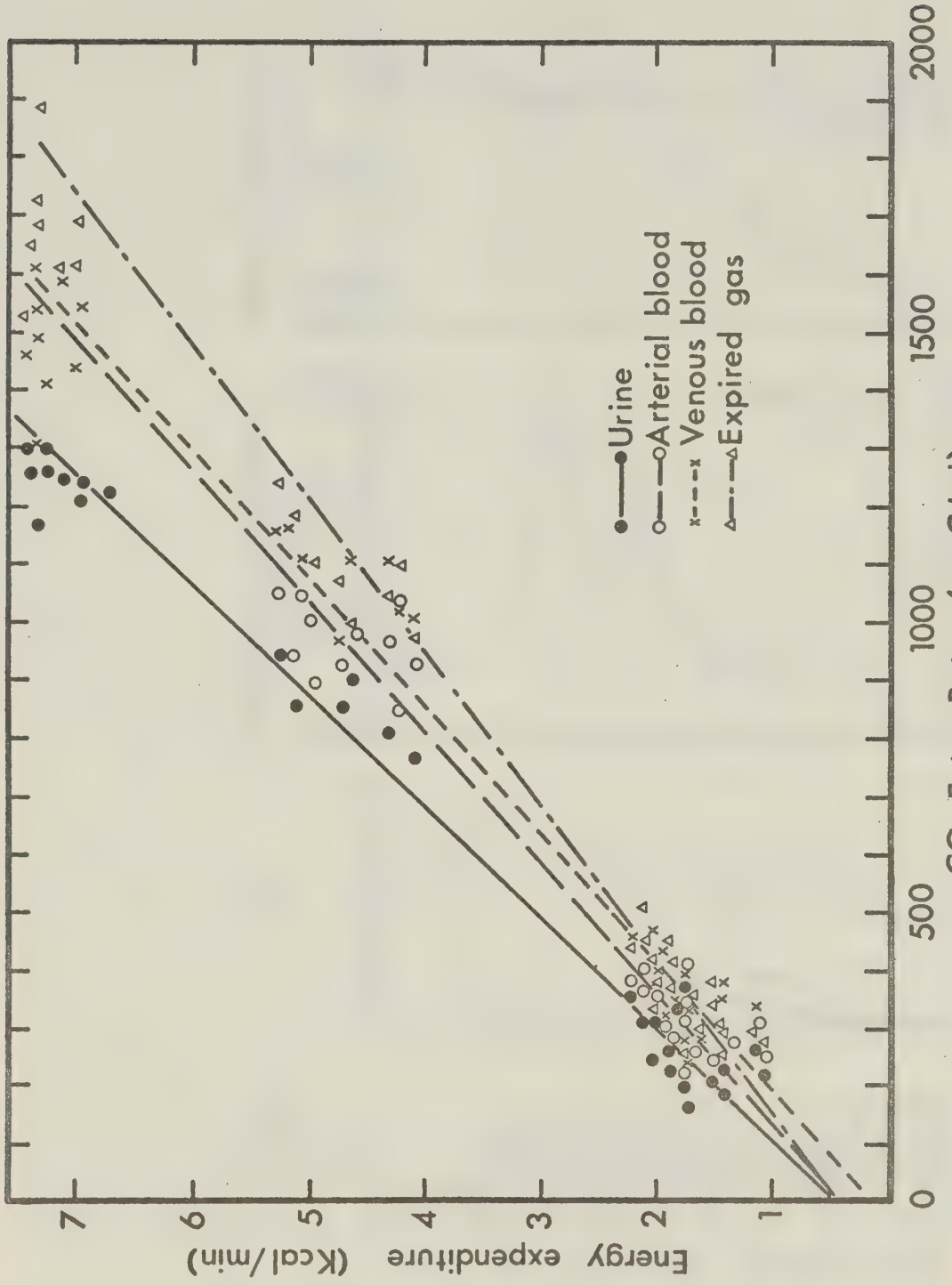


Figure 3. Relationships between rate of energy expenditure estimated from measurements of respiratory gaseous exchanges of sheep and cattle and $CO_2$ entry rate values derived from the body fluids.

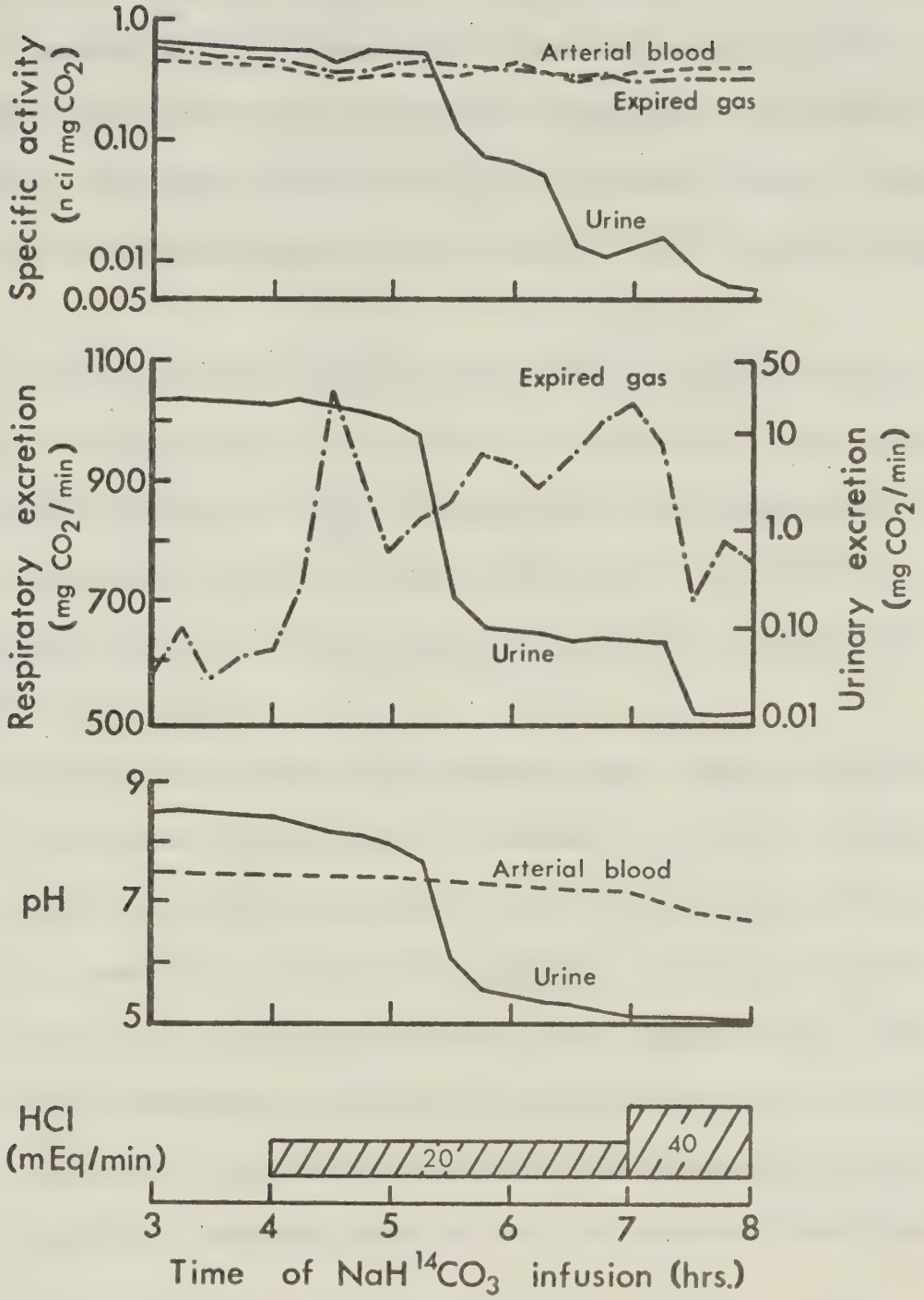


Figure 4. Example of experiment (sheep No. 116; details Appendix B 1) showing effects of intra-ruminal infusion of HCl on pH of body fluids, elimination of $CO_2$ and specific activity of $CO_2$ in the body fluids. Specific activity of $CO_2$ and pH of venous blood were slightly lower than the corresponding arterial bood values.

In the particular results shown in Figure 4 the response to the acid infusion was for the venous and arterial blood pH to decrease slightly. However, urine pH fell from 8.42 to 5.05. The $CO_2$ excretion rate was increased from 585 to 790 mg $CO_2$/min in expired gas and decreased from 23.46 to 0.01 mg $CO_2$/min in urine. In another animal (Sheep 8224, Appendix B 2) the change in expired $CO_2$ was not as marked. The specific activity of $CO_2$ was decreased slightly in arterial blood, venous blood and expired gas but a significant ($p < 0.01$) decrease occurred in urine.

In Figure 5 the results from both trials are used to show the relationship between blood pH and the specific activity of $CO_2$ in body fluids. These results indicated that specific activity of $CO_2$ in venous blood, arterial blood and expired gas were relatively constant in spite of change of blood pH with acidification. However, the specific activity of $CO_2$ in urine fell dramatically in response to decreases in blood and urine pH.

Shortly after the trial on sheep 116 the animal died. After the 420 minute of $NaH^{14}CO_2$ infusion,rate of acid infusion was doubled to 40 m Eq of HCl/min. This increase in rate of acid infusion apparently caused the pH of the arterial and venous blood to decreased to 6.75 and 6.70 respectively. However, the urine pH which was already very low decreased relatively little (Appendix B 1). The excreted rate of urine decreased to one-fifth of that before the acid infusion rate was doubled. Furthermore, red coloration apparently from hemoglobin appeared in the urine. The trial was terminated after 480 min, but the animal died 2 hours later.

C Experiment C Field Measurement

The carbon dioxide entry rate (mg C/min) of seven heifers and cows were estimated from the specific activity of $CO_2$ in urine collected over 24 hours and in

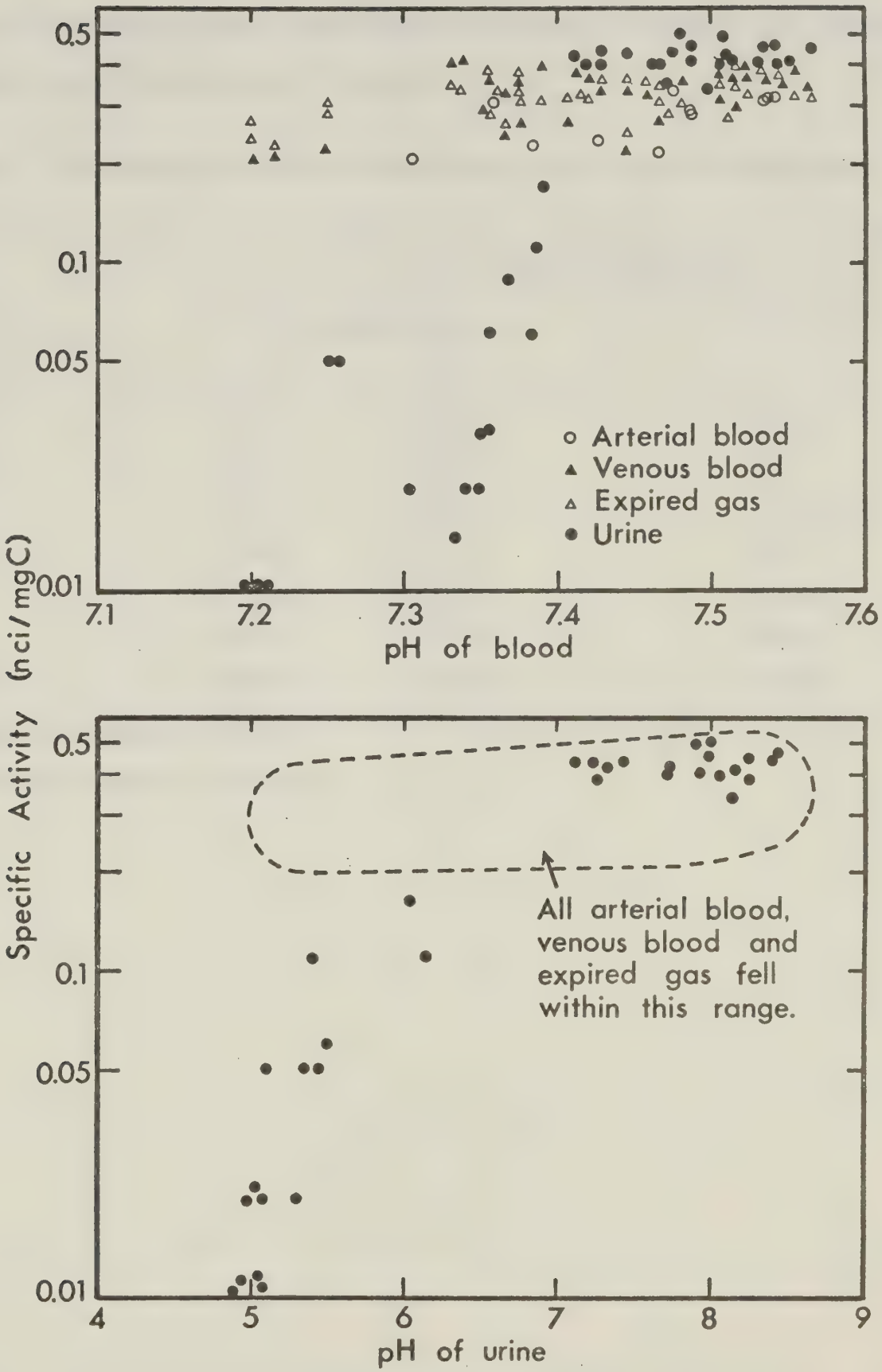


Figure 5. Effect of venous blood and urine pH on specific activity of $CO_2$ in body fluids. Specific activity values were corrected to a standard infusion of 100 n ci/min. For details see Appendix B 1 and B 2.

jugular blood samples taken at the fifth and 29th hour of infusion. The measured entry rate values are shown in Table 6.

Table 6 Measurement of carbon dioxide entry rate from cattle in the field.

| Animal No. | $CO_2$ entry rate (mg C/min) from jugular blood a | b | from urine |
|---|---|---|---|
| 378 | 1901.1 | 2130.1 | 1532.5 |
| 108 | 2007.6 | 1943.2 | 1661.1 |
| 165 | 1937.6 | 1814.5 | 1421.8 |
| 35 | 1742.5 | 2031.2 | 1502.1 |
| 41 | 1912.3 | 2146.0 | 1874.2 |
| 144 | 2021.2 | 1814.0 | 1902.6 |
| 36 | 1643.6 | 1701.0 | 1133.2 |

a transient sample at 5th hour

b transient sample at 29th hour

# DISCUSSION

## A. Equilibrium of Infused $^{14}C$ In Body Pool of $CO_2$

In experiment A, the equilibrium times or times to reach plateau specific activity of $CO_2$ during constant infusion of $NaH^{14}CO_3$ were 3 hours and 4 hours for sheep and cattle, respectively (Figure 2). These observations are similar to those of Huber et al. (1965), Annison et al. (1967 ), Bergman et al. (1967), White et al. (1968), Young et al. (1969) and Corbett et al. (1971) for sheep, and Young (1970) for cattle. The longer time to reach the equilibrium plateau of specific activity of $CO_2$ in cattle was probably a reflection of the greater body mass and thus larger pool of $CO_2$ in cattle.

Kornberg et al. (1952) and Steele (1955) suggested that the body pool of $CO_2$ was divided into three compartments: a central blood compartment, and two peripheral compartments; the soft tissues and bone. During constant infusion of $NaH^{14}CO_3$ into an animal the label is apparently taken up into the soft tissues at varying rates from the blood. The $^{14}C$ content of any tissue will therefore reach a maximum when the specific activity of $CO_2$ in the blood compartment and in the soft tissue compartment reach equilibrium. Not until all body compartments of $CO_2$ have reached equilibrium will a "true plateau" situation exist. Some compartments, eg. bone, will take substantial time to reach equilibrium with the central blood compartment because of the slow flux of $HCO_3^-$ in bone. Buchanan et al. (1951), Bergman and Hougue (1967) found about a 3% variation in the specific activity of expired $CO_2$ during the apparent "plateau" period and suggested that the $HCO_3^-$ in the bone compartment had a much slower flux than that of the soft tissue compartments. Thus after the equilibrium of specific activity of $CO_2$ is reached between the central blood compartment and soft tissue compartments, the equilibrium between the blood compartment and bone compartment may still continue for some time.

Ash et al. (1963) found the rumen epithelium is readily permeable to both $HCO_3^-$ and $CO_2$. Huber et al. (1965) made constant infusion of $NaH^{14}CO_3$ into the sheep via the jugular vein and they found 23% of infused $^{14}C$ in rumen at 4th hour and 4% at 6th hour after beginning infusion. The reason for this large variation is not clear, however, it is apparent that the rumen could contain unequilibriated amounts of $^{14}CO_2$ and might contribute to variations in specific activity of $CO_2$ in the circulatory compartment even after the plateau is reached. $CO_2$ can be fixed in animal tissue (Skipper et al. 1949 and Witternan et al. 1967) and incorporated into the end product of metabolism (eg. methane, urea; Rust et al. 1963). Variations in the rates of these processes could affect the kinetics of $CO_2$ in the animal body and thus lead to the variation of specific activity of $CO_2$ in the body.

In the present study the lack of significant change in specific activity of $CO_2$ with the time after the plateau was reached, except as a consequence of acidosis, indicates that the bone compartment, rumen metabolism and tissue fixation of $CO_2$ apparently did not have major effects.

B. Variation in SA at Different Sites After Plateau Equilibrium

$CO_2$ in venous blood, arterial blood, expired gas and urine are a part of or usually mainly derived directly from the central blood compartment of acid volatile $CO_2$. However, the specific activity of $CO_2$ sampled from these different sites were not the same (Table 5). These differences were probably largely determined by the site of $^{14}C$ infusion and relative local dilution of labelled $CO_2$ by the unlabelled $CO_2$ from cellular metabolism. The explanation might be best illustrated by Figure 6.

In Table 5 it was shown that the specific activity of $CO_2$ in venous blood was on the average 10% lower than that in arterial blood which reflects that considerable amount of unlabelled metabolic $CO_2$ was produced from the tissue and entered the

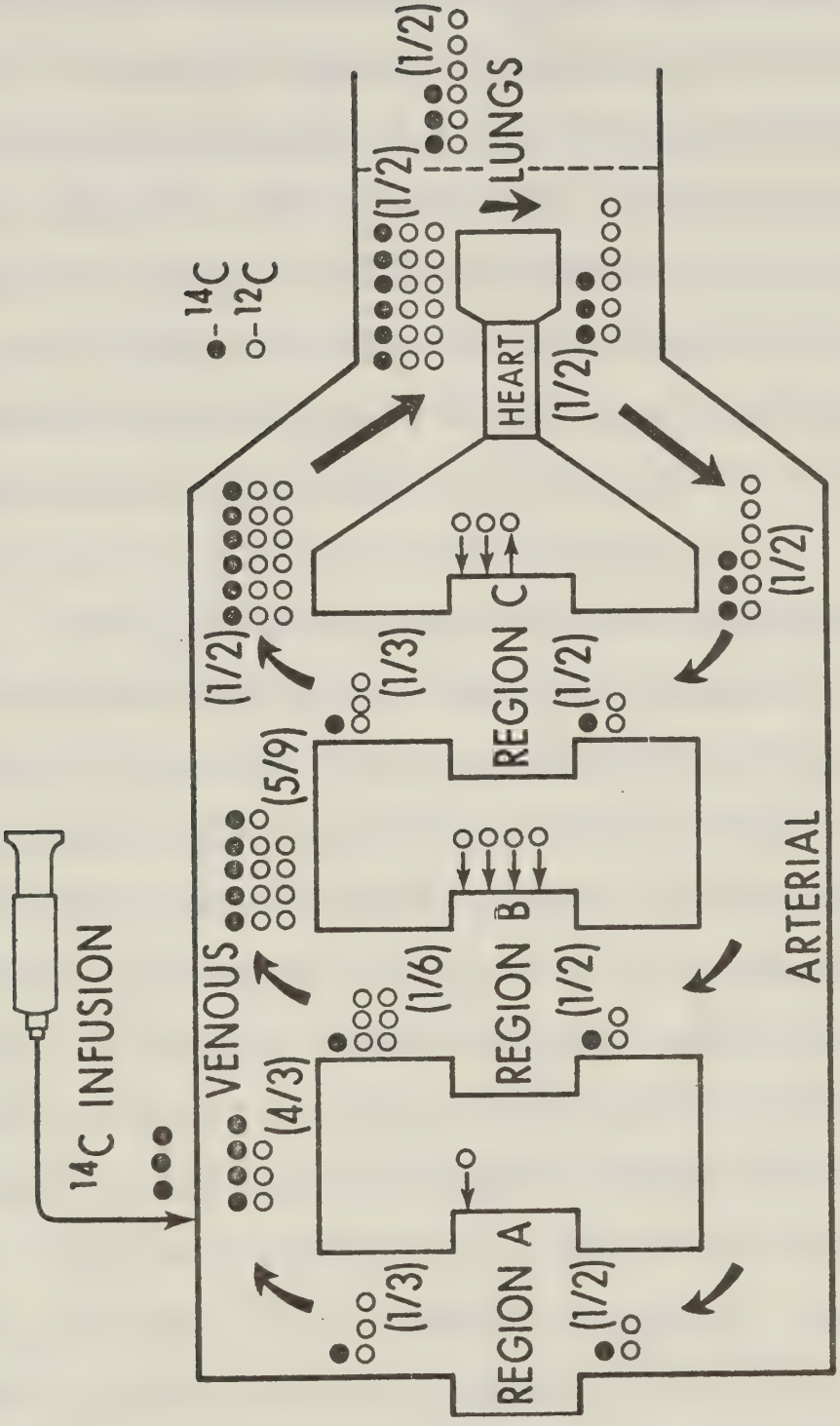


Figure 6. Scheme illustrating effects of local dilution and site of infusion of label on the specific activity of $CO_2$ in the circulating blood compartment.
Region A — low rate of $CO_2$ production
Region B — rapid rate of $CO_2$ production
Region C — production and removal of $CO_2$

blood compartment diluting the labelled $^{14}CO_2$. The specific activity of $CO_2$ in expired gas was on average 6% lower than that in venous blood probably because of dilution by eructated unlabelled $CO_2$ produced by microbial fermentation in the rumen. Furthermore, unlabelled $CO_2$ produced by the lung tissue itself and expired directly without entering the circulatory blood compartment might be the other factor making the specific activity of $CO_2$ in the expired gas generally lower than that of venous blood. In trials on sheep 308 and 48, the specific activity of $CO_2$ in expired gas were on average a little higher than that of venous blood. This apparently high specific activity of $CO_2$ in expired gas may have arisen from infused label entering the rumen without prior mixing with the circulatory blood and then the eructation of the rumen gas with the expired gas.

The $CO_2$ appearing in the urine is usually considered to be that derived from the arterial blood and from metabolically produced $CO_2$ from the urinary organs. However, in the present study the specific activity of $CO_2$ in urine was on average 22% higher than that in arterial blood (Table 5). This result may have arisen because of the site of infusion of $NaH^{14}CO_3$ was near the urinary organs. If the urinary bladder was permeable to both $CO_2$ and $HCO_3^-$, the infused $NaH^{14}CO_3$ may have diffused into the urinary bladder or associated organs and increased the specific activity of $CO_2$ in urine. If such were to occur then some of the label appearing in the urine would never have entered the circulatory blood compartment.

The specific activity of $CO_2$ in rumen fluid and milk were only 59% and 45%, respectively, of that in venous blood (Table 5). These results suggest that the $CO_2$ in rumen fluid and milk were not in free exchange with the circulatory blood $CO_2$ compartment.

As the specific activity of $CO_2$ derived from samples taken from various sites in the body will depend upon locally produced $CO_2$, any $CO_2$ entry rate value based on such samples may differ from the actual entry rate

(turnover rate) of $CO_2$ within the whole animal. The estimations of entry rate derived from the various samples of $CO_2$ might, therefore, not be a precise measurement of the rate of turnover of $CO_2$, but a function of the value. Therefore, as previously suggested by Young et al. (1969) and Corbett et al. (1971) the $CO_2$ entry rate technique really only gives an index of energy expenditure and it is necessary to establish an emperical relationship between the entry rate value and actual rate of energy expenditure.

From the results obtained during experiment A, regression relationships were established between rate of energy expenditure and entry rate values based upon sample of $CO_2$ derived from arterial blood, venous blood, expired gas, and urine.

Young (1968) and Corbett et al. (1971) found that regression equations based upon the specific activity of $CO_2$ in the urine were more precise than those based on $CO_2$ derived from venous blood. In the present study the urine based regression (equation 2) had the highest correlation coefficient. Some of the improved precision with urine samples observed by Young and co-worker probably arose because they sampled blood at 15 min to 30 min intervals, whereas the urine samples accumulated in the bladder over the same period as the measurement of respiratory gaseous exchanges. In addition, these workers suggested that the specific activity of $CO_2$ in urine might be less rapidly affected by transient changes in body $CO_2$ production or output, such as could occur when animals were handled.

## C. Influence of Body pH on Specific Activity of $CO_2$

The kidneys together with the respiratory system have roles in maintaining a normal body pH in animals. These roles are largely through adjustment of the bicarbonate and carbonic acid level in the circulating blood.

With the development of acidosis there was a marked reduction in the specific

activity of $CO_2$ in urine but little change in the specific activity in other body fluids (Table 7). This apparently arose because the specific activity of $CO_2$ in urine is influenced by the increase bicarbonate reabsorption in kidney, including $^{14}C$, during the acidosis condition. Thus the specific activity of $CO_2$ appearing in the urine depends upon a) the amounts of $CO_2$ filtered, b) the amount of $CO_2$ reabsorbed in the renal tubules, c) the diluting capacity of unlabelled $CO_2$ produced from the collecting tubule and urinary bladder and excreted in the urine and d) the transfer of labelled or unlabelled $CO_2$ across the wall of the bladder and associated organs. The rate of glomerular filtration and tubular reabsorption of $CO_2$ is influenced by the pH of the blood and body fluids (Christensen, 1963). Therefore, changes in pH in the body could lead to a change in the specific activity of $CO_2$ in urine. Rector et al. (1965) showed that when animals are in state of acidosis, the renal tubules increased bicarbonate reabsorption by the secretion of hydrogen ion in exchange for sodium ion.

Table 7 Mean of the specific activity of $CO_2$ in body fluids and $CO_2$ elimination rate during alkalosis (normal) and acidosis condition

| Sheep No. | | Mean of specific activity of $CO_2$* (n ci/mg C) | | | | $CO_2$ elimination rate (mg $CO_2$/min) | |
|---|---|---|---|---|---|---|---|
| | | Arterial Blood | Venous Blood | Urine | Expired Gas | Urinary | Respiratory |
| 116 | alkalosis | 0.3784 | 0.3747 | 0.5955 | 0.4466 | 21.524 | 740 |
| | acidosis | 0.3431 | 0.3092 | 0.0315 | 0.3691 | 0.0318 | 897 |
| 8224 | alkalosis | - | 0.1275 | 0.1532 | 0.1233 | 14.195 | 627 |
| | acidosis | - | 0.1193 | 0.0196 | 0.1101 | 0.1282 | 726 |

* Difference in specific activity between alkalosis and acidosis condition were not significant except for urine where the difference was significant at $p < 0.01$. Furthermore, arterial blood, venous blood and expired gas were not significantly different. For original results see Appendix B 1 and B 2.

The acidification of sheep by intra-ruminal infusion of hydrochloric acid, see Experiment B, modified the dynamic of carbon dioxide excretion. A likely mechanism of this modification is illustrated in Figure 7 and explained below.

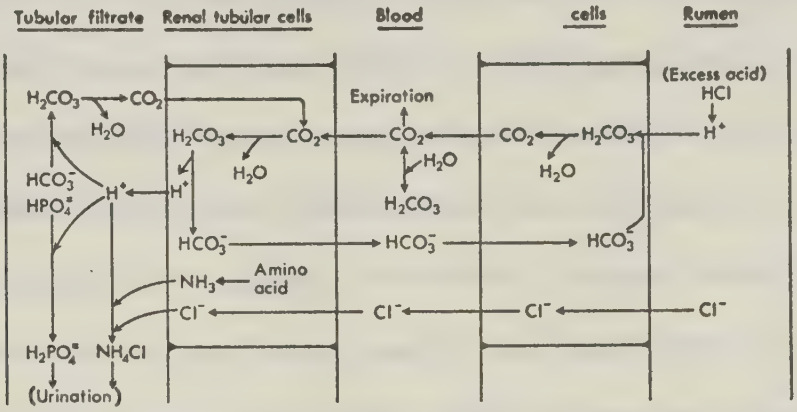


Figure 7. Proposed mechanism of elimination of excess acid and renal tubular reabsorption of bicarbonate following intra-ruminal infusion of HCl.

Excess acid in the rumen is buffered by bicarbonate in cells of the rumen wall to form carbonic acid and neutral salt (NaCl). The carbonic acid then dissociates to form $CO_2$ and water.

Thus:

$$HCl + NaHCO_3 \longrightarrow Na^+Cl^- + H_2CO_3 \longrightarrow H_2O + CO_2$$

The carbon dioxide then diffuses into blood, and is largely eliminated by the respiratory system.

The neutral salt ($Na^+Cl^-$) is transported to the kidney by the blood stream. To prevent excessive loss of sodium ions the anion ($Cl^-$) combined in the kidney with ammonia ($NH_3$) and hydrogen ion ($H^+$) to form ammonium salt ($NH_4Cl$) which is finally excreted in the urine.

Thus:

$$Na^+Cl^- + H_2CO_3 + NH_3 \longrightarrow \underset{\text{(excreted)}}{NH_4Cl} + \underset{\text{(reabsorbed)}}{NaHCO_3}$$

This mechanism agrees with a recent report of Scott et al. for sheep (1970), calves (1971a) and red deer (1971b). Scott and co-workers found that with alkalosis ruminants excreted alkaline urine rich in bicarbonate and poor in ammonium

ion. However, after they infused acid into the rumen the urine became acid, had a low bicarbonate content and a marked increase in ammonium ion concentration.

In the present study the sheep after acidification had little change in their blood pH however the urine pH fell from 8.450 to 4.88 (Figure 4 and 5). The concentration of bicarbonate in the urine was reduced from 866 m Eq/l to 0.02m Eq/l. These indicate the renal reabsorption of bicarbonate was increased when the animals were in a state of acidosis. The regression relationship between urine pH and log urinary bicarbonate excretion for all sheep used in the present study was highly significant ($r = 0.95$, $df = 48$, $p < 0.01$) (Figure 8). Therefore, when an animal becomes acidosis condition the $CO_2$ filtrated from the arterial blood is rapidly reabsorbed back into the circulating blood compartment. Thus during an $NaH^{14}CO_2$ infusion there would be an upsetting of the equilibrium of specific activity of $CO_2$ between urine and blood compartments (Figure 4 and 5 and Table 7). The specific activity of $CO_2$ in urine fell rapidly when the blood pH fell below 7.42. However, the value of specific activity of $CO_2$ in arterial blood, venous blood and expired gas were relatively constant and in equilibrium with each other. Therefore, the carbon dioxide entry rate technique as proposed by Young et al. (1969) and Corbett et al. (1971) could be considered applicable at any pH where the specific activity values were derived from arterial blood, venous blood or expired gas, however, not applicable to urine samples where the urine is acid.

Pitts and Lotspeich (1946) indicated that almost all of the bicarbonate was reabsorbed and return to the blood when sheep were inacid condition and the reabsorption occured in proximal and distal tubules. In the study sheep 116 (Figure 4 and Table 7) there was about 0.0318 mg $CO_2$/min of $CO_2$ excreted in the urine with the acidosis condition. This small amount of $CO_2$ could have arisen from the metabolism of the cells of collecting tubules and urinary bladder. If this were so then it should not contain any label, as all of the labelled carbon filtrated

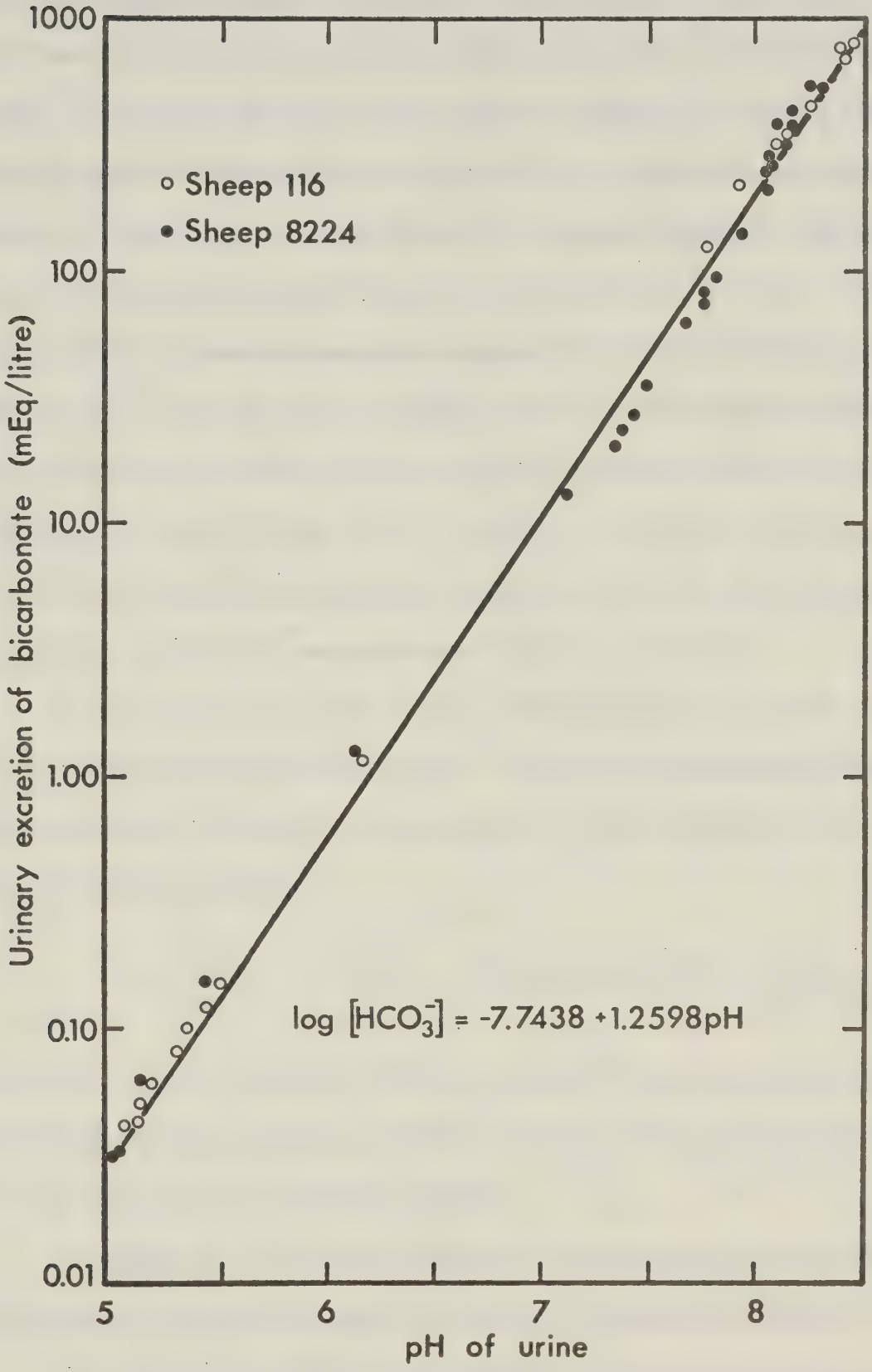


Figure 8. The relationship between urinary pH and urinary bicarbonate excretion in sheep.

from the blood should be reabsorbed and returned to the blood. However, on average 0.0315 n ci/mg C of specific activity of $CO_2$ was found to occur in urine in acidosis condition. This label may have entered the urine not from the filtrate but from infused $^{14}C$ directly diffusing into the collecting tubules and urinary bladder. In alkalosis condition the specific activity of $CO_2$ in urine was on average 0.22 n ci/mg C higher than in arterial blood. As discussed above the high amounts of label in urine may have arisen directly from the diffusion of infused $^{14}C$ through the wall of the bladder and associated organs. The relatively high apparent diffusion of $^{14}C$ into the urine in alkalosis condition might indicate that $CO_2$ or $HCO_3^-$ diffusion through the wall of the urinary bladder or collecting tubules is influenced by the pH of body fluids. Therefore, the cells of collecting tube and urinary bladder may have the similar function to that of the renal tubular cells in regulation of the $HCO_3^-$ excretion and retention in the body.

If it is assumed that with acidosis condition there is complete reabsorption of $CO_2$ by the renal tubules (Pitts et al. 1946) then the excreted unlabelled $CO_2$ (from metabolism) by the collecting tubules and urinary bladder can be estimated using the following formula:

$$\left(1 - \frac{SA_2}{SA_1}\right) \times 100\% \times CO_2 \text{ excreting rate in acidic urine (mg C/min)}$$

where $SA_1$, is specific activity of $CO_2$ (n ci/mg C in urine when the animal in an alkalosis condition and $SA_2$ is specific activity of $CO_2$ (n ci/mg C) in urine when the animal is in an acidosis condition.

For sheep No. 116 it was estimated that the excreted unlabelled $CO_2$ by the collecting tubules and urinary bladder was on average 0.0082 mg C/min.

On the basis of 0.0082 mg of unlabelled carbon per minute excreted by the collecting tubular and urinary bladder cells the $^{14}C$ in filtrate leaving the renal tubules would be diluted about 0.1% for animals in alkalosis, but for the animal

in an acidosis condition it is a 20 fold dilution. Thus the metabolic $CO_2$ excreted from collecting tubule and urinary bladder could markedly upset the equilibrium of specific activity of $CO_2$ in urine when an animal is in an acidosis condition.

The respiratory and urinary appearance of infused $^{14}C$ was markedly affected by changes of pH in the body (Table 8). In acidosis conditions there was more $^{14}C$ recovered in expired gas than when the animal was in alkalosis condition. This probably reflects respiratory system increases eliminate $H_2CO_3$ as $CO_2$ to decrease amounts of $H_2CO_3$ in the blood. In contrast as discussed above there was less $^{14}C$ recovered in the urine in acidosis than in alkalosis condition indicated the kidney increased $HCO_3^-$ reabsorption to increase the $HCO_3^-$ level in the blood.

Table 8 Mean and $\pm$ S E for label elimination rate and percent recovery of infused $NaH^{14}CO_3$ of sheep in alkalosis and acidosis conditions (from Appendix B).

| Sheep No. | | pH | | Label elimination rate (n ci/min) | | Total label recovered (%) | |
|---|---|---|---|---|---|---|---|
| | | Venous Blood | Urine | Urine | Expired gas | Urine | Expired Gas |
| 116 | alkalosis | 7.51-7.40 | 8.4-7.7 | 3.189 $\pm$ 0.388 | 87.75 $\pm$ 2.60 | 2.4 | 65.6 |
| | acidosis | 7.37-7.20 | 6.1-5.0 | 0.001 $\pm$ 0.001 | 93.45 $\pm$ 3.23 | 0.0 | 69.9 |
| 8224 | alkalosis | 7.55-7.42 | 8.2-7.1 | 1.886 $\pm$ 0.210 | 21.07 $\pm$ 0.57 | 3.9 | 59.5 |
| | acidosis | 7.39-7.33 | 6.1-4.8 | 0.009 $\pm$ 0.006 | 22.20 $\pm$ 0.99 | 0.002 | 62.5 |

In the present experiments 59.5 to 69.9% (Table 8) of infused $^{14}C$ was recovered in the expired gas. This is similar to the 52.3 to 65% of recovery reported by Huber et al. (1965) for sheep but was less than the value of 90% recovery reported by Krebs et al. (1951) for mice. The lower recovery of infused $^{14}C$ in the sheep might reflect the relatively large capacity of rumen and retention of $^{14}C$

and subsequently loss by eructation. Hoernicke et al. (1965) estimated that the formation of methane from the $CO_2$ in the rumen could account for about 10% of total carbon dioxide tumover rate in ruminants. Furthermore, some of the infused $^{14}C$-label could be lost from cutaneous diffusion or incorporated into metabolites, for example in the Krebs cycle, urea synthesis and other $CO_2$ fixation process. In the ruminant species in particular, the $CO_2$ also could be incorporated into propionate for gluconeogenesis.

## D. Application of Carbon Dioxide Entry Rate Technique for Estimation Energy Expenditure on Free Ranging Cattle

Rates of energy expenditure shown in Table 9 were calculated from the entry rate values presented in Table 6 and using prediction equation (1) and (2). The estimated daily energy expenditure of heavier, milking cows and the growing heifer were higher than that of lighter weight dry cow. These results illustrate that the carbon entry rate technique could be used in studies on energy metabolism of field animal.

Table 9 Rate of energy expenditure of free ranging cattle estimated by the carbon dioxide entry rate technique.

| Animal No. | Wt. (kg) | Energy Expenditure (Mcal/24h) from jugular blood a* | b* | from urine | |
|---|---|---|---|---|---|
| 378 | 342 | 12.520 | 15.820 | 12.165 | 14 months growing heifer |
| 108 | 341 | 13.210 | 12.793 | 13.128 | " |
| 165 | 296 | 12.757 | 11.959 | 11.336 | " |
| 35 | 342 | 11.493 | 13.363 | 11.937 | 24 months milking cow |
| 41 | 351 | 12.593 | 14.107 | 14.724 | " |
| 144 | 452 | 13.298 | 11.956 | 14.936 | " |
| 36 | 382 | 10.852 | 11.224 | 9.175 | 24 months dry cow |

* same as Table 6.

The variation of estimated energy expenditure from the entry rate of venous blood indicate that transient sampling may not be suitable for field energy expenditure studies over a period of 24 hours because the energy expenditure could be changed substantially within the 24 hours. A method for continuous collection of urine from freely grazing cow was used to obtain a more precise estimation of energy expenditure under field conditions. The method has been previously reported by Young (1970) and further detail of it are given in the Experimental Procedure section. The main disadvantage of this method is that urine is unsuitable as a means of estimating entry rate of $CO_2$ if the animal is in an acidosis condition during any of the measurment period. Another disadvantage is that urinary bladder catheterization or its equivalent, is difficult to establish in the male animals. Farrell et al. (1970) have recently developed equipment for continuous automatic blood sampling in sheep. Such a system could be used for other field animals and may eliminate the problems of transient sampling and acidosis. It is applicable to both female and male animals. This blood sampling system and the $CO_2$ entry rate technique may be a practical and most useful method for the further studies of energy expenditure of unrestrained animals.

## SUMMARY AND CONCLUSION

The index method for estimating energy expenditure based upon measurement of the carbon dioxide entry rate was evaluated for sheep and cattle in alkalosis and acidosis condition. The plateau equilibrium between infused $^{14}CO_2$ and metabolically produced $CO_2$ was established by a constant intraperitoneal infusion of $NaH^{14}CO_3$. Approximately four hours of infusion was needed to reach the plateau in cattle while sheep required approximately three hours. During the plateau equilibrium the specific activity of $CO_2$ had slight variation which were apparently dependent upon the transient variations in metabolic $CO_2$ production and fixation in tissues.

The values of specific activity of $CO_2$ derived from arterial blood, venous blood, expired gas and urine are not the same. These differences probably arose because of local dilution of the infused $^{14}CO_2$ with variable amounts of $CO_2$ produced in the different regions of the body. Regression equations between actual rate of energy expenditure and carbon dioxide entry rate values derived from the arterial blood, venous blood and expired gas were significantly ($p < 0.01$) different. It was concluded that for application of the $CO_2$ entry rate method individual regression equation need to be established for $CO_2$ derived from body fluids taken from different sites in the body.

The specific activity of $CO_2$ appearing in the urine depends upon (a) the amounts of $CO_2$ filtered and reabsorbed in the renal tubules and (b) the diluting capacity of unlabelled $CO_2$ produced from the metabolism of cells of the collecting tubules and urinary bladder. The rate of renal tubular filtration and reabsorption of $CO_2$ from the blood was influenced by the pH of the body fluids. It was observed that after acidification of the animal, by acid infusion into the rumen, the $CO_2$ filtered from the arterial blood in the glomeruli of the kidneys was apparently rapidly reabsorbed back into the blood. Therefore, during an infusion of labelled

$CO_2$ the diluting capacity of unlabelled metabolic $CO_2$ from the collecting tubules and urinary bladder become larger and could reduce the specific activity of $CO_2$ in the urine. The specific activity of $CO_2$ in the arterial blood, venous blood and expired gas were relatively unaffected by a change in pH in the body fluids. Therefore, the carbon dioxide entry rate technique as suggested by Young et al. (1969) and Corbett et al. (1971) could be used in animals in any state of acidosis where the specific activity of $CO_2$ were derived from arterial blood, venous blood or expired gas, but not from urine sample when the urine is acid (pH $>$ 7).

The carbon dioxide entry rate method was used to measure the rate of energy expenditure of seven free ranging beef cattle. The daily energy expenditures of heavier, milking cows and the growing heifers were higher than that of lighter and dry cows. These results confirmed that the carbon dioxide entry rate method could be a useful and convenient tool in studies on the energy metabolism of free ranging animals with the exception that urine should not be used to derive $CO_2$ for specific activity analysis when the animal's urine is acid.

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# APPENDIX A

Specific activity of carbon dioxide in various body fluids during the intraperitoneal infusion of $NaH^{14}CO_3$ and energy expenditure estimated from respiratory gaseous exchange.

Appendix A 1

Sheep No. 48 Infusion Rate 25.5 n ci/min

| Time of Infusion | Specific Activity of $CO_2$ (n ci/min) Arterial Blood | Venous Blood | Urine | Expired Gas | $O_2$ Consumption (l/min) | $CO_2$ Production (l/min) | Energy Expenditure Kcal/min) |
|---|---|---|---|---|---|---|---|
| 000 | 0 | 0 | 0 | 0 | - | - | - |
| 030 | 0.0404 | 0.0372 | 0.0432 | 0.0324 | - | - | - |
| 060 | 0.0578 | 0.0523 | 0.0660 | 0.0652 | - | - | - |
| 090 | 0.0632 | 0.0664 | 0.0771 | 0.0643 | - | - | - |
| 120 | 0.0813 | 0.0732 | 0.0829 | 0.0774 | - | - | - |
| 150 | 0.0892 | 0.0874 | 0.0907 | 0.0809 | - | - | - |
| 180 | 0.1127 | 0.1132 | 0.1052 | 0.1059 | 0.3813 | 0.3850 | 1.936 |
| 210 | 0.1032 | 0.1088 | 0.1077 | 0.1042 | 0.3621 | 0.4336 | 1.920 |
| 240 | 0.1272 | 0.1048 | 0.1249 | 0.0897 | 0.3241 | 0.4556 | 1.823 |
| 270 | 0.1237 | 0.1008 | 0.1262 | 0.0811 | 0.3214 | 0.4407 | 1.771 |
| 300 | 0.1081 | 0.1145 | 0.1113 | 0.0810 | 0.3312 | 0.4568 | 1.827 |
| 330 | 0.1128 | 0.1122 | 0.1226 | 0.0876 | 0.3240 | 0.4375 | 1.777 |
| 360 | 0.1264 | 0.1172 | 0.1198 | 0.0862 | 0.3171 | 0.4168 | 1.726 |
| 390 | 0.1072 | 0.1044 | 0.1032 | 0.0708 | 0.3532 | 0.4204 | 1.869 |
| 420 | 0.1068 | 0.0992 | 0.1137 | 0.0742 | 0.3754 | 0.4310 | 1.968 |
| mean* | 0.1142 [a] | 0.1089 [ab] | 0.1149 [ab] | 0.0867 | | | |
| S E (±) | 0.0050 | 0.0023 | 0.0029 | 0.0040 | | | |

* mean estimated from 180 to 420 min and the same letters indicated not significantly different at $p < 0.05$.

Appendix A 2

Sheep No. 146 Infusion Rate 52.6 n ci/min

| Time of Infusion | pH | | | Specific Activity of $CO_2$ (n ci/mgC) | | | | $O_2$ | $CO_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Arterial Blood | Venous Blood | Urine | Arterial Blood | Venous Blood | Urine | Expired Gas | Consumption (l/min) | Production (l/min) | Energy Expenditure (Kcal/min) |
| 180 | 7.67 | 7.68 | 8.10 | 0.1285 | 0.1110 | 0.1396 | 0.1204 | 0.3940 | 0.5068 | 2.1318 |
| 210 | 7.67 | 7.65 | 8.23 | 0.1378 | 0.1125 | 0.1476 | 0.1164 | 0.3992 | 0.5480 | 2.2010 |
| 240 | 7.65 | 7.63 | 8.22 | 0.1668 | 0.1225 | 0.1532 | 0.1222 | 0.3671 | 0.4240 | 1.9280 |
| 270 | 7.59 | 7.54 | 8.25 | 0.1649 | 0.1538 | 0.1601 | 0.1392 | 0.3421 | 0.3965 | 1.7983 |
| 300 | 7.60 | 7.58 | 8.48 | 0.1884 | 0.1556 | 0.1688 | 0.1300 | 0.3507 | 0.4220 | 1.8622 |
| 330 | 7.64 | 7.60 | 8.45 | 0.1903 | 0.1584 | 0.1909 | 0.1351 | 0.3403 | 0.3390 | 1.7234 |
| 360 | 7.67 | 7.65 | 8.49 | 0.1280 | 0.1298 | 0.2137 | 0.1551 | 0.3412 | 0.3369 | 1.7233 |
| 390 | 7.50 | 7.45 | 8.48 | 0.1908 | 0.1872 | 0.1978 | 0.1805 | 0.3301 | 0.2760 | 1.6073 |
| mean* | | | | a 0.1613 | ab 0.1414 | a 0.1714 | b 0.1322 | | | |
| S E (±) | | | | 0.0095 | 0.0094 | 0.0091 | 0.0080 | | | |

* mean followed the same letters are not significantly different at $p < 0.05$.

Appendix A 3

Sheep No. 8224 Infusion Rate 69.5 n ci/min

| Time of Infusion | pH | | | Specific Activity of $CO_2$ (n ci/mg C) | | | | $O_2$ | $CO_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Arterial Blood | Venous Blood | Urine | Arterial Blood | Venous Blood | Urine | Expired Gas | Consumption (l/min) | Production (l/min) | Energy Expenditure (Kcal/min) |
| 180 | 7.670 | 7.608 | 8.36 | 0.1634 | 0.1989 | 0.3431 | 0.1855 | 0.3789 | 0.3105 | 1.8415 |
| 210 | 7.648 | 7.623 | 8.38 | 0.2261 | 0.2037 | 0.3839 | 0.2393 | 0.3717 | 0.2891 | 1.7962 |
| 240 | 7.590 | 7.642 | 8.16 | 0.1869 | 0.1870 | 0.2584 | 0.1755 | 0.4050 | 0.2805 | 1.9059 |
| 270 | 7.632 | 7.684 | 8.26 | 0.2785 | 0.2047 | 0.3142 | 0.1616 | 0.3878 | 0.2531 | 1.8039 |
| 300 | 7.670 | 7.666 | 8.07 | 0.1895 | 0.1852 | 0.2759 | 0.1648 | 0.4183 | 0.2642 | 1.9374 |
| 330 | 7.690 | 7.632 | 8.17 | 0.1875 | 0.1838 | 0.2292 | 0.1480 | 0.4698 | 0.2933 | 2.1682 |
| 360 | 7.678 | 7.670 | 8.32 | 0.2223 | 0.1776 | 0.3170 | 0.1600 | 0.4219 | 0.2518 | 1.9405 |
| 390 | 7.640 | 7.572 | 8.28 | 0.2353 | 0.2143 | 0.3839 | 0.2861 | 0.2857 | 0.2778 | 1.4380 |
| mean * | | | | a 0.2104 | ab 0.2026 | 0.3143 | b 0.1884 | | | |
| S E (±) | | | | 0.0134 | 0.0079 | 0.0240 | 0.0173 | | | |

* mean followed the same letters are not significantly different at $p < 0.05$.

Appendix A 4

Sheep No. 308 — Infusion Rate 53.3 n ci/min

| Time of Infusion | pH | | | Specific Activity of $CO_2$ (n ci/mg C) | | | | $O_2$ | $CO_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Arterial Blood | Venous Blood | Urine | Arterial Blood | Venous Blood | Urine | Expired Gas | Consumption (l/min) | Production (l/min) | Energy Expenditure (Kcal/min) |
| 210 | 7.59 | 7.55 | 8.11 | 0.2128 | 0.1568 | 0.1750 | 0.1117 | 0.2059 | 0.1778 | 1.0094 |
| 240 | 7.59 | 7.57 | 8.32 | 0.2149 | 0.1999 | 0.2552 | 0.0931 | 0.2227 | 0.1862 | 1.0475 |
| 270 | 7.59 | 7.50 | 8.37 | 0.2112 | 0.1695 | 0.2656 | 0.1401 | 0.3119 | 0.2515 | 1.5077 |
| 300 | 7.60 | 7.55 | 8.41 | 0.1732 | 0.1918 | 0.2033 | 0.1490 | 0.3708 | 0.3762 | 1.8867 |
| 330 | 7.60 | 7.64 | 8.35 | 0.2305 | 0.1880 | 0.2931 | 0.1736 | 0.3720 | 0.2983 | 1.7961 |
| 360 | 7.57 | 7.59 | 8.30 | 0.2532 | 0.1978 | 0.2619 | 0.1874 | 0.3620 | 0.3263 | 1.7968 |
| 390 | 7.50 | 7.42 | 8.25 | 0.1864 | 0.2176 | 0.3227 | 0.1926 | 0.3547 | 0.2884 | 1.7097 |
| 420 | 7.55 | 7.45 | 8.25 | 0.1409 | 0.1859 | 0.2384 | 0.2247 | 0.3671 | 0.2991 | 1.7781 |
| 450 | 7.62 | 7.40 | 8.25 | 0.1401 | 0.1659 | 0.2690 | 0.1817 | 0.3567 | 0.2986 | 1.7374 |
| 480 | 7.59 | 7.54 | 8.25 | 0.1995 | 0.1376 | 0.2723 | 0.1670 | 0.3151 | 0.2597 | 1.4168 |
| 510 | 7.54 | 7.48 | 8.20 | 0.1719 | 0.1571 | 0.1931 | 0.1783 | 0.3127 | 0.2701 | 1.1552 |
| mean * | | | | 0.1939 | 0.1631 b | 0.2454 | 0.1653 b | | | |
| S E (±) | | | | 0.0107 | 0.0090 | 0.0106 | 0.0103 | | | |

* mean followed the same letters are not significantly different at $p < 0.05$.

Appendix A 5

Sheep No. 48 — Infusion Rate 63.1 n ci/min

| Time of Infusion | pH | | | Specific Activity of $CO_2$ (n ci/mg C) | | | | $O_2$ | $CO_2$ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Arterial Blood | Venous Blood | Urine | Arterial Blood | Venous Blood | Urine | Expired Gas | Consumption (l/min) | Production (l/min) | Energy Expenditure (Kcal/min) |
| 180 | 7.48 | 7.45 | 8.11 | 0.2094 | 0.1897 | 0.2759 | 0.1990 | 0.3921 | 0.3258 | 1.9068 |
| 210 | 7.54 | 7.53 | 8.10 | 0.1840 | 0.1632 | 0.2636 | 0.1845 | 0.4070 | 0.3302 | 1.9666 |
| 240 | 5.52 | 7.50 | 8.12 | 0.2609 | 0.1968 | 0.2912 | 0.2000 | 0.3792 | 0.3158 | 1.9653 |
| 270 | 7.55 | 7.52 | 8.15 | 0.1633 | 0.1571 | 0.2654 | 0.1782 | 0.4173 | 0.3468 | 2.0281 |
| 300 | 7.61 | 7.57 | 8.13 | 0.1758 | 0.1359 | 0.2545 | 0.1687 | 0.4093 | 0.3438 | 1.9950 |
| 330 | 7.58 | 7.56 | 8.30 | 0.2039 | 0.1804 | 0.2743 | 0.1826 | 0.3960 | 0.3248 | 1.9210 |
| 360 | 7.59 | 7.55 | 8.25 | 0.1533 | 0.1626 | 0.2068 | 0.1601 | 0.4151 | 0.3525 | 2.0219 |
| 390 | 7.62 | 7.60 | 8.32 | 0.2093 | 0.1772 | 0.2618 | 0.1930 | 0.3976 | 0.3447 | 1.9315 |
| 420 | 7.58 | 7.58 | 8.48 | 0.2318 | 0.1920 | 0.3143 | 0.1992 | 0.3849 | 0.3343 | 1.8894 |
| 450 | 7.64 | 7.61 | 8.45 | 0.1990 | 0.1735 | 0.2335 | 0.1814 | 0.4011 | 0.3546 | 1.9762 |
| mean * | | | | a 0.1990 | b 0.1728 | 0.2641 | ab 0.1846 | | | |
| S E (±) | | | | 0.0101 | 0.0056 | 0.0091 | 0.0041 | | | |

* mean followed the same letters are not significantly different at $p < 0.05$.

Cattle No. 3 Infusion Rate 171.3 n ci/min

| Time of Infusion | Specific Activity of $CO_2$ (n ci/ mg C) | | | | $O_2$ Consumption (l/min) | $CO_2$ Production (l/min) | Energy Expenditure (Kcal/min) |
|---|---|---|---|---|---|---|---|
| | Arterial Blood | Venous Blood | Urine | Expired Gas | | | |
| 000 | 0 | 0 | 0 | 0 | - | - | - |
| 030 | 0.0513 | 0.0371 | 0.0545 | 0.0472 | - | - | - |
| 060 | 0.0823 | 0.0723 | 0.1110 | 0.0643 | - | - | - |
| 090 | 0.1004 | 0.0925 | 0.1336 | 0.0837 | - | - | - |
| 120 | 0.1186 | 0.1092 | 0.1379 | 0.1072 | - | - | - |
| 150 | 0.1203 | 0.1173 | 0.1413 | 0.1201 | - | - | - |
| 180 | 0.1343 | 0.1314 | 0.1703 | 0.1137 | - | - | - |
| 210 | 0.1426 | 0.1378 | 0.1674 | 0.1301 | - | - | - |
| 240 | 0.1642 | 0.1543 | 0.1978 | 0.1445 | 0.996 | 1.033 | 5.090 |
| 270 | 0.1625 | 0.1469 | 0.1815 | 0.1377 | 1.042 | 1.042 | 5.278 |
| 300 | 0.1699 | 0.1710 | 0.1911 | 0.1536 | 0.977 | 0.982 | 4.956 |
| 330 | 0.1695 | 0.1458 | 0.2084 | 0.1436 | 1.023 | 1.035 | 5.190 |
| 360 | 0.1813 | 0.1519 | 0.1903 | 0.1710 | 0.912 | 0.904 | 4.610 |
| 390 | 0.1777 | 0.1486 | 0.2117 | 0.1620 | 0.857 | 0.845 | 4.326 |
| 420 | 0.1839 | 0.1765 | 0.2010 | 0.1601 | 0.930 | 0.948 | 4.733 |
| 450 | 0.1678 | 0.1665 | 0.2018 | 0.1560 | 0.848 | 0.842 | 4.232 |
| 480 | 0.1835 | 0.1703 | 0.2227 | 0.1756 | 0.801 | 0.824 | 4.085 |
| mean * | 0.1729 | 0.1591 [b] | 0.2007 | 0.1560 [b] | | | |
| S E (±) | 0.0026 | 0.0039 | 0.0041 | 0.0042 | | | |

* mean are estimated from 240 to 480 min. and the followed same letter are not significant different at $p < 0.05$.

Appendix A 7

Cattle No. 32

Infusion Rate 221.0 n ci/min

| Time of Infusion | Specific Activity of $CO_2$ (n ci/mg C) | | | | | $O_2$ Consumption (l/min) | $CO_2$ Production (l/min) | Energy Expenditure (Kcal/min) |
|---|---|---|---|---|---|---|---|---|
| | Venous Blood | Urine | Expired Gas | Rumen Fluid | Milk | | | |
| 255 | 0.1571 | 0.1709 | 0.1446 | 0.0857 | 0.0744 | 1.568 | 1.121 | 7.407 |
| 270 | 0.1432 | 0.1775 | 0.1336 | 0.0887 | 0.0652 | 1.558 | 1.123 | 7.371 |
| 285 | 0.1372 | 0.1793 | 0.1282 | 0.0893 | 0.0923 | 1.556 | 1.095 | 7.329 |
| 300 | 0.1623 | 0.1875 | 0.1079 | 0.0627 | 0.0652 | 1.523 | 1.075 | 7.299 |
| 315 | 0.1526 | 0.1763 | 0.1379 | 0.0615 | 0.0923 | 1.496 | 1.069 | 7.019 |
| 330 | 0.1432 | 0.1802 | 0.1375 | 0.0836 | 0.0789 | 1.487 | 1.032 | 6.944 |
| 345 | 0.1521 | 0.1813 | 0.1049 | 0.0999 | 0.0697 | 1.422 | 1.048 | 6.755 |
| 360 | 0.1443 | 0.1743 | 0.1130 | 0.1039 | 0.0620 | 1.540 | 1.126 | 7.297 |
| 375 | 0.1392 | 0.1774 | 0.1296 | 0.1019 | 0.0539 | 1.503 | 1.123 | 7.166 |
| 390 | 0.1478 | 0.1694 | 0.1312 | 0.0995 | 0.0507 | 1.691 | 1.113 | 7.300 |
| 405 | 0.1572 | 0.1708 | 0.1401 | 0.0842 | 0.0552 | 1.519 | 1.145 | 7.246 |
| 420 | 0.1542 | 0.1812 | 0.1307 | 0.0994 | 0.0673 | 1.466 | 1.055 | 6.934 |
| means * | 0.1491 | 0.1708 | 0.1282 | 0.0887 | 0.0681 | | | |
| SE (±) | 0.0022 | 0.0015 | 0.0037 | 0.0041 | 0.0036 | | | |

* all the means are significantly different to each other at $p < 0.05$.

# APPENDIX B

Specific activity of $CO_2$ in various body fluids during intraperitoneal infusion of $NaH^{14}CO_3$ and intra-ruminal infusion of HCl, and the effection of $CO_2$ elimination by acid infusion.

Appendix B 1

## Sheep No. 116

## Infusion Rate 133.65 n ci/min

| Time of $^{14}C$ Infusion | pH | | | Specific Activity of $CO_2$ (n ci/mg C) | | | | Urine Excretion | | | | Respiratory Gas | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Arterial Blood | Venous Blood | Urine | Arterial Blood | Venous Blood | Urine | Expired Gas | Volume (L) | $PCO_2$ (mm Hg) | $HCO_3^-$ (m Eq/L) | Total $CO_2$ (m Eq/L) | $O_2$ Consumption (L/min S.T.P.) | $CO_2$ Production (L/min S.T.P.) | Energy Expenditure (Kcal/min) |
| 180 | 7.532 | 7.512 | 8.42 | 0.4306 | 0.4038 | 0.6530 | 0.5349 | 0.01 | 127 | 796.0 | 799.8 | 0.2382 | 0.2981 | 1.2789 |
| 195 | - | - | 8.45 | - | - | 0.6421 | 0.5490 | 0.01 | 129 | 866.4 | 870.2 | 0.2397 | 0.3356 | 1.3293 |
| 210 | 7.540 | 7.503 | 8.44 | 0.4285 | 0.4306 | 0.6378 | 0.4771 | 0.01 | 125 | 820.4 | 824.1 | 0.2592 | 0.2916 | 1.3579 |
| 225 | - | - | 8.43 | - | - | 0.6426 | 0.4686 | 0.01 | 130 | 833.8 | 837.7 | 0.2453 | 0.3128 | 1.3236 |
| *240 | 7.484 | 7.465 | 8.43 | 0.4080 | 0.3796 | 0.5805 | 0.4685 | 0.01 | 120 | 769.6 | 773.2 | 0.2527 | 0.3201 | 1.3610 |
| 255 | - | - | 8.25 | - | - | 0.5693 | 0.4186 | 0.01 | 112 | 474.6 | 477.9 | 0.3260 | 0.3758 | 1.7112 |
| 270 | 7.464 | 7.446 | 8.17 | 0.2970 | 0.2978 | 0.4776 | 0.3346 | 0.02 | 108 | 380.6 | 383.9 | 0.3531 | 0.5352 | 2.0072 |
| 285 | - | - | 8.10 | - | - | 0.6363 | 0.3359 | 0.02 | 110 | 330.0 | 333.3 | 0.3188 | 0.4698 | 1.7961 |
| 300 | 7.427 | 7.405 | 7.95 | 0.3287 | 0.3622 | 0.5575 | 0.4314 | 0.02 | 105 | 223.0 | 226.1 | 0.2747 | 0.3972 | 1.5385 |
| 315 | - | - | 7.75 | - | - | 0.5583 | 0.4480 | 0.03 | 95 | 127.3 | 130.1 | 0.2993 | 0.4315 | 1.6748 |
| 330 | 7.382 | 7.375 | 6.15 | 0.3084 | 0.3584 | 0.1510 | 0.4169 | 0.03 | 35.5 | 1.19 | 2.3 | 0.2961 | 0.4406 | 1.6734 |
| 345 | - | - | 5.50 | - | - | 0.6788 | 0.4176 | 0.04 | 20.5 | 0.15 | 0.77 | 0.3058 | 0.4795 | 1.7576 |
| 360 | 7.352 | 7.250 | 5.85 | 0.4331 | 0.3110 | 0.6720 | 0.6041 | 0.04 | 20 | 0.13 | 0.73 | 0.3043 | 0.4770 | 1.7488 |
| 375 | - | - | 5.35 | - | - | 0.0703 | 0.3956 | 0.04 | 19.5 | 0.10 | 0.69 | 0.3076 | 0.4510 | 1.7303 |
| 380 | 7.305 | 7.215 | 5.30 | 0.2918 | 0.2886 | 0.0289 | 0.3176 | 0.04 | 17 | 0.08 | 0.59 | 0.3466 | 0.4821 | 1.9181 |
| 405 | - | - | 5.20 | - | - | 0.0146 | 0.3203 | 0.04 | 18.5 | 0.06 | 0.62 | 0.3564 | 0.5106 | 1.9905 |
| **420 | 7.106 | 7.200 | 5.12 | 0.3392 | 0.2789 | 0.0116 | 0.3193 | 0.04 | 15.5 | 0.05 | 0.51 | 0.3576 | 0.5268 | 2.0145 |
| 435 | - | - | | - | - | 0.0180 | 0.3187 | 0.04 | 17.5 | 0.04 | 0.57 | 0.3188 | 0.4911 | 1.8217 |
| 450 | 6.950 | 6.852 | 5.10 | 0.4173 | 0.3519 | 0.0084 | 0.3577 | 0.008 | 16.5 | 0.05 | 0.54 | 0.2738 | 0.4023 | 1.5413 |
| 465 | - | - | 5.05 | - | - | 0.0065 | 0.3229 | 0.008 | 14.5 | 0.04 | 0.47 | 0.2524 | 0.3537 | 1.4001 |
| 480 | 6.750 | 6.700 | 5.08 | 0.3606 | 0.2822 | 0.0061 | 0.3596 | 0.008 | 15.5 | 0.04 | 0.50 | 0.2920 | 0.4088 | 1.6193 |

* Acid infusion 20 m Eq/min of HCl from 240 min to 420 min

** Acid infusion 40 m Eq/min of HCl from 420 min to 480 min

Appendix B 2

## Sheep No. 8224

## Infusion Rate 35.3 n ci/min

| Time of $^{14}C$ Infusion | pH | | Specific Activity of $CO_2$ (n ci/mg C) | | | Urine Excretion | | | | Respiratory Gas | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Venous Blood | Urine | Venous Blood | Urine | Expired Gas | Volume (L) | $PCO_2$ (mm Hg) | $HCO_3^-$ (m Eq/L) | Total $CO_2$ (m Eq/L)[2] | $O_2$ Consumption (L/min S.T.P.) | $CO_2$ Production (L/min S.T.P.) | Energy Expenditure (Kcal/min) |
| 180 | 7.550 | 8.12 | 0.1407 | 0.1498 | 0.1222 | 0.02 | 110 | 345.5 | 384.8 | 0.4828 | 0.3102 | 2.4710 |
| 195 | 7.545 | 8.22 | 0.1239 | 0.1403 | 0.1314 | 0.02 | 115 | 454.7 | 458.2 | 0.3109 | 0.3203 | 1.5920 |
| 210 | 7.542 | 8.26 | 0.1204 | 0.1327 | 0.1077 | 0.02 | 126 | 546.3 | 550.1 | 0.2755 | 0.3327 | 1.4215 |
| 225 | 7.562 | 8.26 | 0.1218 | 0.1556 | 0.1180 | 0.02 | 130 | 563.7 | 567.6 | 0.3048 | 0.3561 | 1.5667 |
| *240 | 7.542 | 8.15 | 0.1222 | 0.1484 | 0.1283 | 0.02 | 105 | 353.4 | 356.5 | 0.3081 | 0.3478 | 1.5584 |
| 255 | 7.535 | 8.06 | 0.1336 | 0.1470 | 0.1455 | 0.02 | 96 | 262.6 | 265.5 | 0.3296 | 0.3314 | 1.6874 |
| 270 | 7.521 | 8.08 | 0.1369 | 0.1311 | 0.1170 | 0.02 | 110 | 315.1 | 318.4 | 0.3431 | 0.3128 | 1.7381 |
| 285 | 7.512 | 8.09 | 0.1403 | 0.1507 | 0.1243 | 0.025 | 95.6 | 280.2 | 283.1 | 0.4050 | 0.3536 | 2.0419 |
| 300 | 7.508 | 8.09 | 0.1372 | 0.1509 | 0.1388 | 0.025 | 90 | 263.8 | 266.5 | 0.4252 | 0.3105 | 2.1200 |
| 315 | 7.510 | 8.02 | 0.1507 | 0.1769 | 0.1021 | 0.025 | 80 | 199.6 | 202.0 | 0.3462 | 0.2832 | 1.6976 |
| 330 | 7.482 | 7.92 | 0.1301 | 0.1863 | 0.1085 | 0.025 | 75 | 148.6 | 150.9 | 0.2835 | 0.3118 | 1.4224 |
| 345 | 7.495 | 7.80 | 0.1432 | 0.1793 | 0.1087 | 0.025 | 65.5 | 98.4 | 100.4 | 0.2781 | 0.3800 | 1.4136 |
| 360 | 7.474 | 7.72 | 0.1239 | 0.1568 | 0.1042 | 0.03 | 66 | 82.5 | 84.5 | 0.3416 | 0.3605 | 1.6867 |
| 375 | 7.460 | 7.72 | 0.1134 | 0.1419 | 0.1230 | 0.03 | 62.5 | 78.1 | 80.0 | 0.4320 | 0.3051 | 2.1027 |
| 390 | 7.462 | 7.71 | 0.1179 | 0.1354 | 0.1140 | 0.03 | 64 | 78.2 | 80.1 | 0.4955 | 0.2821 | 2.3716 |
| 405 | 7.440 | 7.45 | 0.1218 | 0.1561 | 0.1303 | 0.03 | 59 | 39.6 | 41.3 | 0.2686 | 0.2720 | 1.4125 |
| 420 | 7.428 | 7.42 | 0.1222 | 0.1557 | 0.1245 | 0.04 | 43.5 | 27.2 | 28.5 | 0.2636 | 0.2997 | 1.4022 |
| 435 | 7.425 | 7.38 | 0.0888 | 0.1542 | 0.1400 | 0.04 | 43.5 | 24.8 | 26.1 | 0.2554 | 0.3061 | 1.3325 |
| 450 | 7.420 | 7.32 | 0.1336 | 0.1502 | 0.1401 | 0.04 | 42. | 20.9 | 22.1 | 0.2769 | 0.3237 | 1.4430 |
| 465 | 7.418 | 7.12 | 0.1369 | 0.1521 | 0.1170 | 0.04 | 41.6 | 13.0 | 14.3 | 0.3315 | 0.2977 | 1.7058 |
| 480 | 7.390 | 6.12 | 0.1219 | 0.0623 | 0.1000 | 0.04 | 38.3 | 1.20 | 2.3 | 0.2800 | 0.3251 | 1.4577 |
| 495 | 7.375 | 5.40 | 0.1340 | 0.0332 | 0.1019 | 0.04 | 27.1 | 0.16 | 0.9 | 0.2937 | 0.3968 | 1.5196 |
| 510 | 7.365 | 5.08 | 0.1223 | 0.0207 | 0.0974 | 0.04 | 22.0 | 0.06 | 0.7 | 0.3405 | 0.3969 | 1.7309 |
| 525 | 7.360 | 5.02 | 0.0917 | 0.0109 | 0.1156 | 0.05 | 13.5 | 0.03 | 0.4 | 0.4051 | 0.3431 | 2.0391 |
| 540 | 7.362 | 4.97 | 0.1045 | 0.0109 | 0.1047 | 0.05 | 12.2 | 0.02 | 0.4 | 0.3581 | 0.3444 | 1.8117 |
| 555 | 7.355 | 4.92 | 0.1206 | 0.0073 | 0.1205 | 0.05 | 12.5 | 0.02 | 0.4 | 0.3234 | 0.3601 | 1.6494 |
| 570 | 7.350 | 4.90 | 0.1194 | 0.0063 | 0.1245 | 0.05 | 15.5 | 0.03 | 0.5 | 0.3350 | 0.4032 | 1.6866 |
| 585 | 7.330 | 4.85 | 0.1225 | 0.0054 | 0.1302 | 0.05 | 12.2 | 0.02 | 0.4 | 0.3182 | 0.3836 | 1.6023 |

* Acid infusion 20 m Eq/min of HCl from 240 to 585 min.